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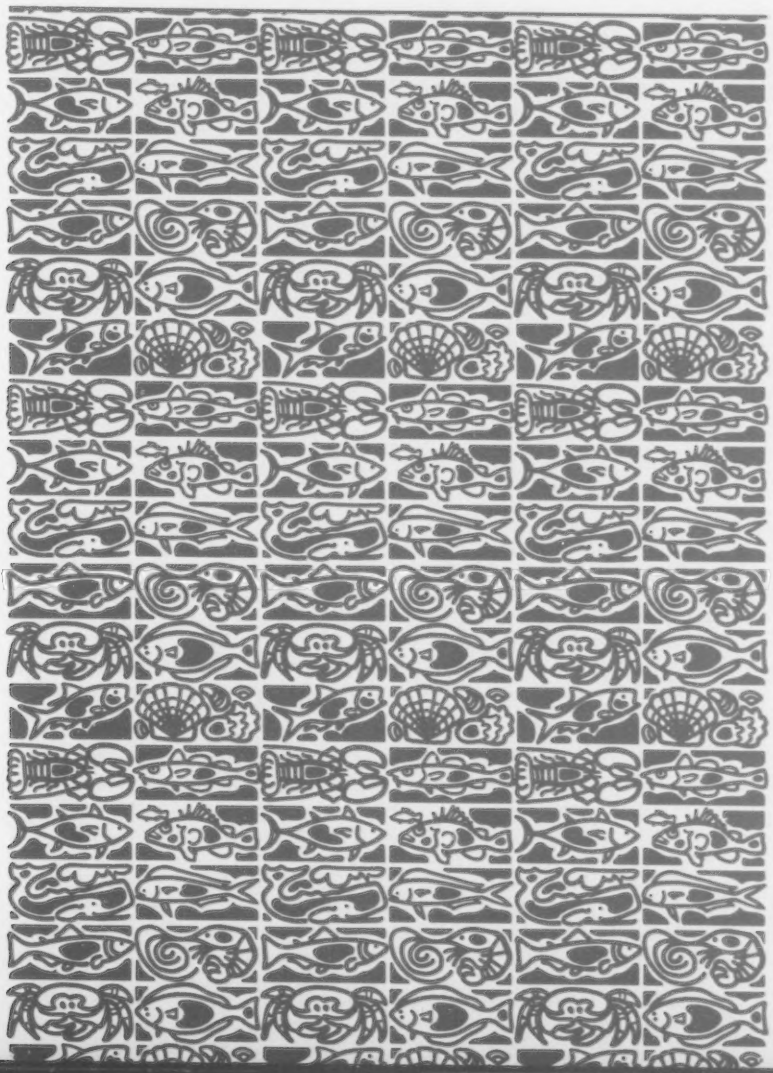

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Fishery Bulletin

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Southeast Fisheries Science Center
National Marine Fisheries Service, NOAA
101 Pivers Island Road
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National Marine Fisheries Service
Scientific Publications Office
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Intrapopulation structure of winter-spawned Argentine shortfin squid, *Illex argentinus* (Cephalopoda, Ommastrephidae), during its feeding period over the Patagonian Shelf

Alexander I. Arkhipkin

Atlantic Research Institute of Marine Fisheries and Oceanography (AtlantNIRO);
5 Dm. Donskoy street, Kaliningrad, 236000 Russia
Present address: Fisheries Department, Falkland Islands Government
PO Box 598, Stanley, Falkland Islands
E-mail address: fish.fig@horizon.co.fk

Abstract.—Stock structure dynamics of the important commercial squid *Illex argentinus* were studied by using biological data from about 25 thousand squid caught January–April 1991 by Russian trawlers in three fishery regions: 51–52°S; 47–49°S within the exclusive economic zone of Argentina (EEZA); and 45–47°S outside the EEZA. A total of 2664 statoliths were read to prepare age-length keys for each 10-day interval of the period studied. It was found that between January and April, the Patagonian shelf south of 45°S was a feeding ground of two intraspecific groups of winter-hatched *I. argentinus*: a shelf group that matured at medium sizes (ShG) and a slope group that matured at large sizes (SlG). After massive immigration of *I. argentinus* from the north in January–February into the two fishery regions within 45–49°S, the stock structure remained rather stable until April, composed predominantly of June- and July-hatched squid. Squid grow and mature rapidly, and males mature at younger ages (from one to two months) than do females. During feeding, some redistribution of the stock was observed: maturing and mature SlG squid (mainly females) tended to shift from the shelf (130–150 m depth) in a northeast direction and concentrate over the shelf edge (160–170 m depth). In April, mature SlG squid began to shift to the continental slope around 45–47°S and migrated to depths >600 m where they then mixed with schools of SlG squid that had fed in the region 51–52°S and that were already migrating northwards along the slope. ShG squid remained on the shelf and made their prespawning northward migrations along the shelf edge.

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The Argentine shortfin squid, *Illex argentinus* (de Castellanos, 1960), is a common neritic species occurring in waters off Brazil, Uruguay, Argentina, and the Falkland Islands in the Southwest Atlantic (Nesis, 1987). This squid is an important world fishery resource. According to the FAO (1997), since 1978, its total annual catch has varied from 180 to 250 thousand metric tons (t), achieving 300–330 thousand tons in 1993–95. However, actual total annual catch of *I. argentinus* could reach up to 700 thousand tons (Uozumi and Shiba, 1993). *Illex argentinus* is captured by the international fleet consisting of both jigging light vessels (mainly from Asian countries) and trawlers (mostly from European countries: Poland, Spain, and Russia (former USSR) in two fishery regions off the Argentine Exclusive Economic Zone (EEZA): at 42°S and 45–47°S. In the 1970s and 1990s, *I. argentinus* was also caught in considerable numbers within the EEZA and Falkland Islands Interim Conservation Zone (FICZ) (Csirke, 1987; FAO, 1997). Such an extensive fishery has induced detailed studies of different biological aspects of *I. argentinus* in order to monitor and forecast its stock structure dynamics.

Originally, *I. argentinus* was considered to be a single stock (Sato and Hatanaka, 1983; Csirke, 1987). Then it was found that the species consisted of two populations differing both by season and place of their spawning: an abundant winter-spawning population (more than 95% of the total stock) and a small summer-spawning population (Hatanaka et al., 1985; Hatanaka, 1988). Brunetti (1988) divided winter-spawning squid into two stocks: the bonaerensis north Patagonian stock (BNPS) and the south Patagonian stock (SPS), differing both by feeding grounds and size of adults (medium and large, respectively). On the basis of occurrence of mature females in different seasons, Nigmatullin (1989a) revealed that *I. argentinus* spawn throughout the year, and proposed to subdivide the total stock into four seasonal spawning groups. Although Tsygankov (1987) found qualitative differences in three loci of esterases extracted from the buccal muscles of various intrapopulational groups of *I. argentinus*, the taxonomic status of these groups, however, still remained unclear. Analysis of dynamics in length-frequency compositions showed that completion of the life cycle of *I. argentinus* populations took one year (Hatanaka et al., 1985; Hatanaka, 1986) and this length of time was then confirmed by statolith aging investigations (Arkhipkin, 1990; Rodhouse and Hatfield, 1990).

The life cycle of the most abundant winter-spawned group can be subdivided into five stages: a postlarval

period that takes place in waters off Brazil and Uruguay in August–September (Leta, 1987; Santos and Haimovici, 1997); a juvenile period that takes place in shelf and oceanic waters off Uruguay and Argentina in September–December (Brunetti, 1988; Parfeniuk et al., 1992); a feeding period that takes place on the Patagonian and Falkland Islands shelves in January–April (Brunetti, 1988; Hatanaka, 1988); a prespawning period that takes place on the shelf edge and slope off Argentina and Uruguay in May–July (Hatanaka, 1986, 1988; Arkhipkin, 1993); and a spawning period that takes place in shelf and slope waters off northern Argentina, Uruguay, and Brazil in July–August (Brunetti, 1988; Santos and Haimovici, 1997). Squid aggregate and are fished mainly during their feeding period on the shelf, as well as during their prespawning period on the shelf edge and slope (Nigmatullin, 1989b).

Stock structure dynamics of *I. argentinus* during their feeding period on the Patagonian Shelf were studied both in the fishery region of 45–47°S outside the EEZA and within the FICZ by using data obtained from Japanese jigging vessels (Rodhouse and Hatfield, 1990; Uozumi and Shiba, 1993). It was found that the age composition of *I. argentinus* catches changed between January and April owing to the gradual migrations of feeding schools of these squid. Earlier hatched squid immigrated to and emigrated from the fishery region earlier than later hatched groups (Uozumi and Shiba, 1993). Squid of the former group had slower growth rates than those of the latter group (Rodhouse and Hatfield, 1990). Data from trawling vessels showed that, during the prespawning period, winter-spawned *I. argentinus* made active northward migrations from the southern Patagonian Shelf along the continental slope of Argentina. Squid migrated in waves of abundance, consisting of 2–4 successive monthly generations. Males moved 2–3 weeks earlier than females of the same monthly group (Arkhipkin, 1993).

An analysis of length-frequency distributions of *I. argentinus* showed that the jigging fishery had a higher selectivity for squid than did the trawl fishery (Koronkiewicz, 1995), and therefore data from the trawl fishery reflected the natural population distribution far better than those from the jigging fishery. In the present report I examined the stock structure dynamics of *I. argentinus* during the January–April feeding period, using both data from two research vessels and two commercial trawlers (both fishing within the fishery) and statolith aging techniques, and comparing these stock structure data with those obtained by the Japanese jigging fishery both within and outside the EEZA. Together with the data obtained during the prespawning period (April–June) (Arkhipkin, 1993), the results of the present study make it possible to reconstruct the full picture of the stock structure dynamics of *I. argentinus* during the entire fishery period.

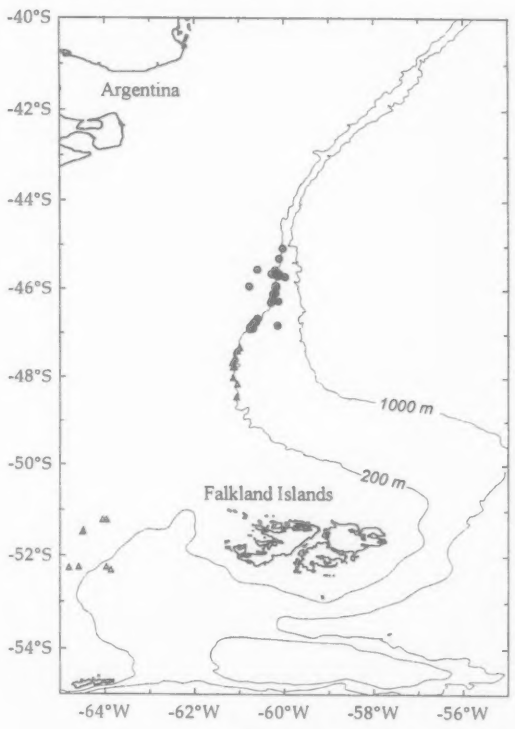

Figure 1

Sampling locations of *Illex argentinus* off (circles) and within (triangles) the Exclusive Economic Zone of Argentina (EEZA) in the southwest Atlantic in January–April 1991.

Materials and methods

Data on the Argentine shortfin squid, *Illex argentinus*, for the present study were collected during four experimental surveys carried out in the fishery region of 45–47°S outside the EEZA by the Soviet research vessels *Anchar* and *Volzhanin* (2700 GRT) and within the EEZA (in 47–49°S and 51–52°S) by the fishing trawlers *Petropavlovskaya krepost* and *Batiliman* (4000 gross registered tonnage [GRT]) between January and April 1991 (Fig. 1). Trawls were conducted with different types of rope trawls with a mean horizontal opening of 60–75 m and mean vertical opening of 40–50 m. Trawls were made in the superficial water layer at night and near bottom in the daytime at bottom depths ranging from 140 to 190 m in January–March

and from 190 to 660 m in April. The duration of trawls ranged from 4 to 8 hours, and the average towing speed was 6–8 km/h.

Every ten days, average daily catch per unit of effort (CPUE) was calculated as a mean of daily CPUEs of the Soviet fishing trawlers that caught squid in each fishery region. Mean CPUEs were calculated separately for the fishery region of 45–47°S outside the EEZA (where a majority of fishing vessels are 2700-GRT trawlers) and for the fishery regions within the EEZA (where all fishing vessels were 4000-GRT trawlers). To obtain an objective picture of the squid fishery, CPUEs of the 2700 GRT trawlers may be adjusted to those of 4000-GRT trawlers by a coefficient of 0.7 (Arkhipkin, 1993).

Length-frequency sampling

A random sample of one hundred squid was taken by scientific observers from each of two catches (at night and day) everyday on board each of the four vessels. Dorsal mantle length (ML) was measured to the nearest 1 mm, total body weight (BW) was weighed to the nearest 1 g. Sex and maturity stages were identified according to the maturity scale elaborated for *Illex argentinus* (Nigmatullin, 1989a). Sex ratio was determined. A total of 16,436 squid were analyzed in the fishery region of 45–47°S outside the EEZA and 9893 squid were analyzed within the EEZA. Every ten days, three length-frequency curves of males and females were constructed for three maturity periods: immature (maturity stages 1–2), maturing (maturity stages 3–5_1) and mature (maturity stages 5_2–5_3).

Age sampling and statolith processing

Every ten days, from January to April, statoliths were dissected from 100–150 individuals of *Illex argentinus* from two successful catches on board each of the four vessels. The length-frequency distribution of the 10-day age sample was proportional to the length-frequency distribution of squid caught during these ten days. Statoliths were washed in distilled water and stored in oil-paper envelopes in 96% ethanol. A total of 1700 statoliths were sampled in the fishery region of 45–47°S outside the EEZA, and 1150 statoliths were collected within the EEZA.

All statoliths sampled were processed by statolith aging techniques in the Laboratory of Commercial Invertebrates of AtlantNIRO (Arkhipkin, 1991). Statolith terminology follows Clarke (1978) and Lipinski et al. (1991). Statoliths were attached to the microscopic slides with Pro-texx mounting medium and were ground on both sides on a wet waterproof sandpaper of 1000-grit grade. During grinding, the statolith rostrum was completely removed, so that growth increments could be easily distinguished from the nucleus to the edge of the dorsal dome. Ground statoliths were embedded in Canada balsam and covered with glass covers. Ready preparations were placed in an oven at 90–100°C for one hour to dry the balsam and improve the readability of growth increments. Statoliths were read under a Biolam R1 light microscope at 450–500× magnification. To avoid possible counting errors, each statolith was counted twice by two observers using the gradation of an eye-piece micrometer. The total number of growth increments for each specimen was obtained as a mean of these replicate counts if the deviation between the two counts was less than 5%. If deviation exceeded 5%, the statolith was recounted by the two observers once more. If such deviation did not decrease after the recounting, the statolith was rejected from further analysis. From the whole sample, 1597 statoliths from the region outside the EEZA (93.9%) and 1067 statoliths from the region within the EEZA (92.7%) were prepared and read.

Length-at-age data analysis

Deposition of putative growth increments within *I. argentinus* statoliths has not yet been validated. However, incorporation of either tetracycline or strontium marks into statoliths of the congeneric species *I. illecebrosus* kept in captivity has shown that growth increments are formed daily (Dawe et al., 1985). Statolith microstructure in both species is similar; therefore growth increments within statoliths of *I. argentinus* are considered to form daily in the present paper. Hence, their total number was considered to represent squid age in days. Hatching dates were backcalculated. Month classes of hatching were defined by pooling squid into each month of hatching (Arkhipkin, 1990; Rodhouse and Hatfield, 1990). Length-at-age data were analyzed separately for both sexes. The 10-day age structure was determined by construction of age-length keys. Age-length keys were constructed by using numbers of squid for each month class separately for each sex and maturity period (Arkhipkin et al., 1996).

Results

CPUE dynamics

Region 45–47°S *Illex argentinus* were caught in all trawls of the research vessels (Fig. 2). In January, schools of squid aggregated mainly north of the region. Trawl catches were variable (from 1 to 20 t per vessel day, t/d), and mean January CPUEs were low (8–9 t/d).

During the first 10-day period of February, concentrations of *I. argentinus* were observed in all parts of the region. Squid concentrated near the bottom during the daytime and ascended to the upper water layers at night. The CPUE was twice as high as in January, and the squid fishery stabilized at 16–17 t/d. However, aggregations of *I. argentinus* quickly dispersed, and during the second 10-day period of February, the CPUE fell sharply to the January level (Fig. 2). Catches of squid were low but stable (around 10 t) until the second 10-day period in April, when fishing vessels shifted from the shelf edge (170–190 m depth) to the continental slope (440–660 m depth) and changed fishing tactics. The vessels performed near-bottom trawls at the shelf edge during the daytime, and on the continental slope at night. These tactics considerably increased CPUE (up to 15–18 t/d) for the third 10-day period of April and in the beginning of May because the fleet began to target not only shelf aggregations but also the slope aggregations of *I. argentinus* that were beginning to appear at that time.

Region 47–49°S Fishing trawlers operated in this region between February and the second 10-day period of March. The fishing tactics were the same as those used in the previous region. Abundance of *I. argentinus* was considerably greater than that in the region outside the EEZA (Fig. 2). The peak of CPUE was observed in the third 10-day period of February (51 t/d).

Region 51–52°S All fishing vessels that had operated in the 47–49°S region moved to the fishing region west of the Falkland Islands during the second 10-day period of March and fished there until the middle of May (Fig. 2). The fishing tactics were different from those used in the two previous regions. Trawlers fished for squid in midwater both at night and in the daytime. CPUE in March–April was high, similar to those in the 47–49°S region with a prominent peak (53 t) during the second 10-day period of April. At the beginning of May, CPUE decreased sharply and the fleet ceased fishing for squid in the region.

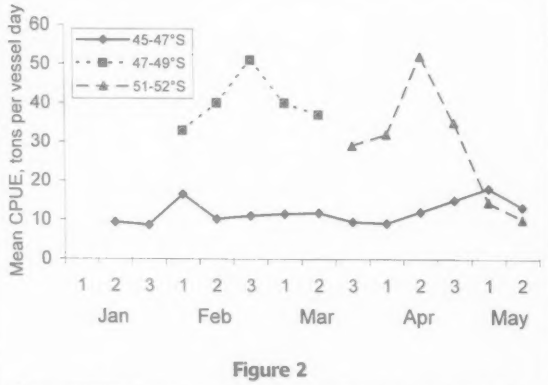

Figure 2

Mean CPUE (tons per vessel day) of the Russian trawlers in the *Illex argentinus* fishery in three fishery regions of the Southwest Atlantic in 1991: 45–47°S (bold solid line), 47–49°S (dotted line) and 51–52°S (dashed line).

Sex ratios

Region 45–47°S The proportion of females was the highest during the second 10-day period of January (ca 80% of the total sample). From the end of January through the beginning of February, the proportion of females decreased sharply (to 55–60%). Sex ratio was close to 1:1 between the second 10-day period of February and the first 10-day period of April, when the proportion of females occurring on the shelf edge decreased to 30%. However during the third 10-day period of April on the continental slope at depths of 480 m, the sex ratio was found to be close to 1:1, and females prevailed in catches (65%) at deeper depths (630 m) (Fig. 3).

Region 47–49°S Except the first 10-day period of February (when the sex ratio was close to 1:1), males always predominated in catches at a ratio of 2:1 (Fig. 3).

Region 51–52°S The sex ratio was close to 1:1, but the proportion of females tended to decrease from 55% to 45% (Fig. 3).

Length and hatching-month composition

Region 45–47°S During the second 10-day period of January, June-hatched maturing females (modal sizes 210 mm ML) and mature males (200 mm) predominated in catches. The proportion of mature May-hatched squid was low. Hatching-month composition changed considerably during the third 10-day period of January owing to an appearance of maturing and mature May-hatched squid (males of 200 mm and females of 220–250 mm ML) in the region (Fig. 4).

Massive appearances of dense schools of immigrating June and July-hatched immature and maturing females (200–220 mm ML) and mature males (200–210 mm ML) were observed in the first 10-day period of February which resulted in a double increase in CPUE of the fishing fleet (Fig. 2) and in corresponding changes in the hatching-month compositions of squid: June-hatched squid

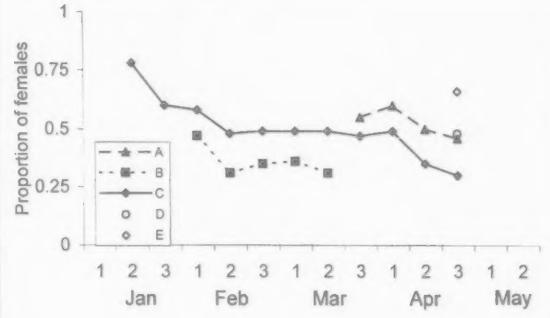

Figure 3

Proportion of females in sex ratio of *Illex argentinus* in three fishery regions of the Southwest Atlantic in 1991: (A) 51–52°S; (B) 47–49°S; and (C–E) 45–47°S. Depths for the last region were 160–190 m (C), 480 m (D), and 630 m (E).

became the most abundant in catches, as in the middle of January. During the second and third 10-day periods of February, hatching-month composition was the same as that of January, with June- and July-hatched squid predominating. However during the third 10-day period, the number of large mature females (280 mm ML) increased considerably (Fig. 4).

In March, the hatching-month composition of the *I. argentinus* catch was approximately similar to that of the second 10-day period of February; June- and July- hatched squid were the most abundant. The proportion of July-hatched squid increased by the end of March with a corresponding decrease in June-hatched squid. Modal sizes of males increased from 220–230 mm ML in the beginning to 230–240 mm ML at the end of the month. Length composition of females was bimodal (240–250 and 290–300 mm ML). The proportion of immature females decreased, whereas the proportion of mature females increased by the end of March (Fig. 5).

In April, hatching-month composition of *I. argentinus* caught over the shelf edge (170–190 m depths) remained almost similar to that of the second and third 10-day periods of March; July-hatched squid were predominant. Almost all males were mature. Immature females disappeared from catches in the first and second 10-day periods of April, but were caught in small numbers in the third 10-day period. At the beginning of the month, the length composition of both maturing and mature females was bimodal (260–270 and 300 mm ML). During the second 10-day period, large maturing and mature females (310 mm ML) began concentrating over the shelf edge, whereas medium-size females (270–280 mm ML) were still dispersed (Fig. 6). These concentrations caused another increase in CPUE for the fishing fleet (Fig. 2). During the third 10-day period of April, large mature squid (females of 310–320 mm ML and males of 270 mm ML) appeared in deeper waters over the continental slope, and they became most abundant at 480–630 m depths. Medium-size squid (maturing females of 280 mm ML and mature males of 250 mm ML) remained over the shelf edge (Fig. 6).

Region 47–49°S During the first 10-day period of February, the length composition of males was unimodal (230 mm ML) and most of these males were mature. Among females, two different modal groups occurred in the catches: immature June-hatched females (220 mm ML) and maturing and mature April- and May-hatched females (260 mm ML). Large catches of June- and July-hatched squid were evident between the second 10-day period of February and first 10-day period of March (Fig. 2). Hatching-month compositions did not change significantly in this period, June- and July-hatched squid were caught almost in equal proportions. Mature males increased slightly in length from 230 to 240 mm ML. The proportion of immature females decreased and that of maturing and mature females increased by the second 10-day period of March (Fig. 7).

Region 51–52°S Hatching-month composition was similar between the third 10-day period of March and second 10-day period of April; July-hatched males and females were predominant in catches. Length compositions were unimodal for both sexes. Except during the third 10-day period of March when about a third of males were maturing, most of the males were mature, and their sizes increased from 260 mm ML at the end of March to 280 mm ML at the end of April. Females grew more rapidly in length than did males (from 280 to 320 mm ML). They matured quickly; immature females prevailed at the end of March, whereas maturing females were predominant at the end of April. During the third 10-day period of April, age composition of *I. argentinus* changed owing to a high proportion of August-hatched squid (Fig. 8).

Comparative comments

Simultaneous sampling in the regions of 45–47°S and 47–49°S between February and March and in the regions of 45–47°S and 51–52°S between March and April enabled a comparison of both length and hatching-month compositions of *I. argentinus* in these regions.

Hatching-month compositions were almost similar in the regions of 45–47°S and 47–49°S during the same 10-day periods (Figs. 4, 5, and 7). Modal sizes

of mature males were about 10–20 mm greater in the southern region than those in the northern region. During the first 10-day period of February, the proportion of large mature females was much higher at 47–49°S (Fig. 7) than at 45–47°S (Fig. 4). After the second 10-day period of February, the opposite situa-

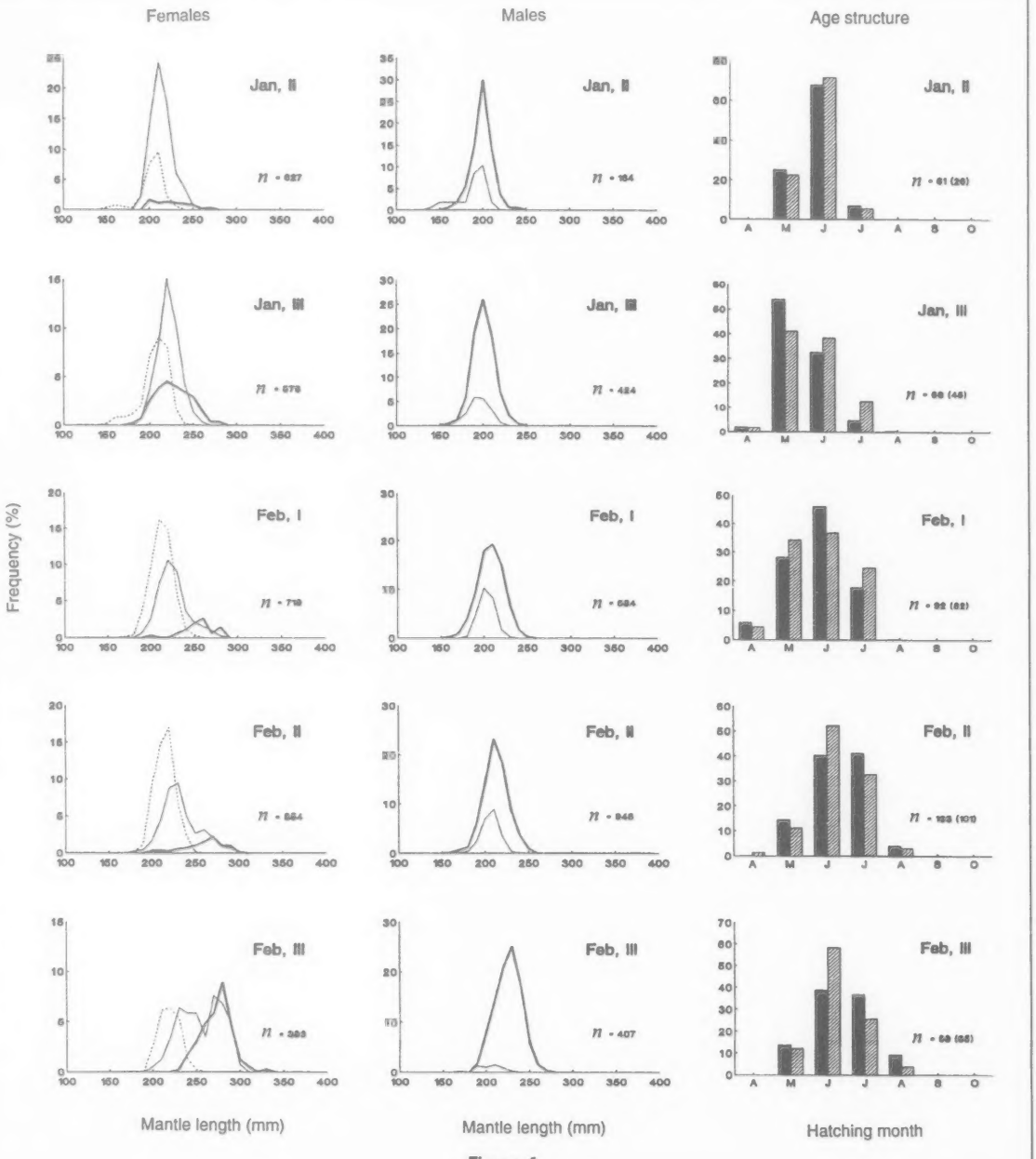

Figure 4

Length-frequency compositions of immature (dotted line), maturing (solid line) and mature (bold solid line) squid and hatching month compositions (age structures) of females (black bars) and males (dashed bars) of *Illex argentinus* in the fishery region of 45–47°S outside the EEZA in January–February 1991. Number of males in parentheses.

tion was observed; the proportion of mature females decreased at 47–49°S and increased at 45–47°S.

Both the length and hatching-month compositions of *I. argentinus* catches were different over the Patagonian Shelf at 45–47°S and 51–52°S. In the southern region, squid were 20–30 mm larger, about a month younger, and less mature than in the northern region, except during the third 10-day period of April when the

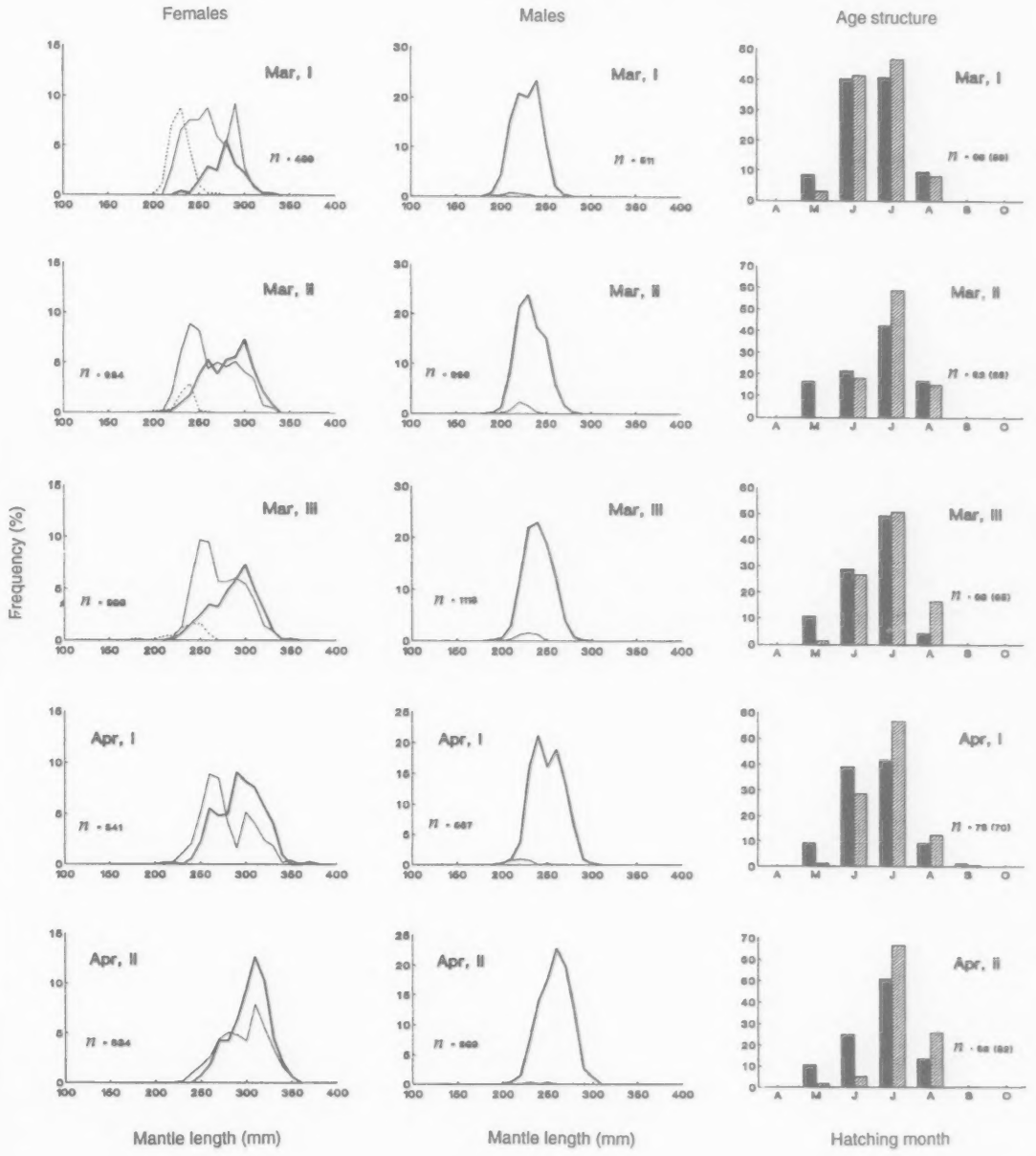

Figure 5

Length-frequency and hatching month compositions (age structures) of *Illex argentinus* in the fishery region of 45–47°S outside the EEZA in March–April 1991. Symbols are the same as in Figure 4.

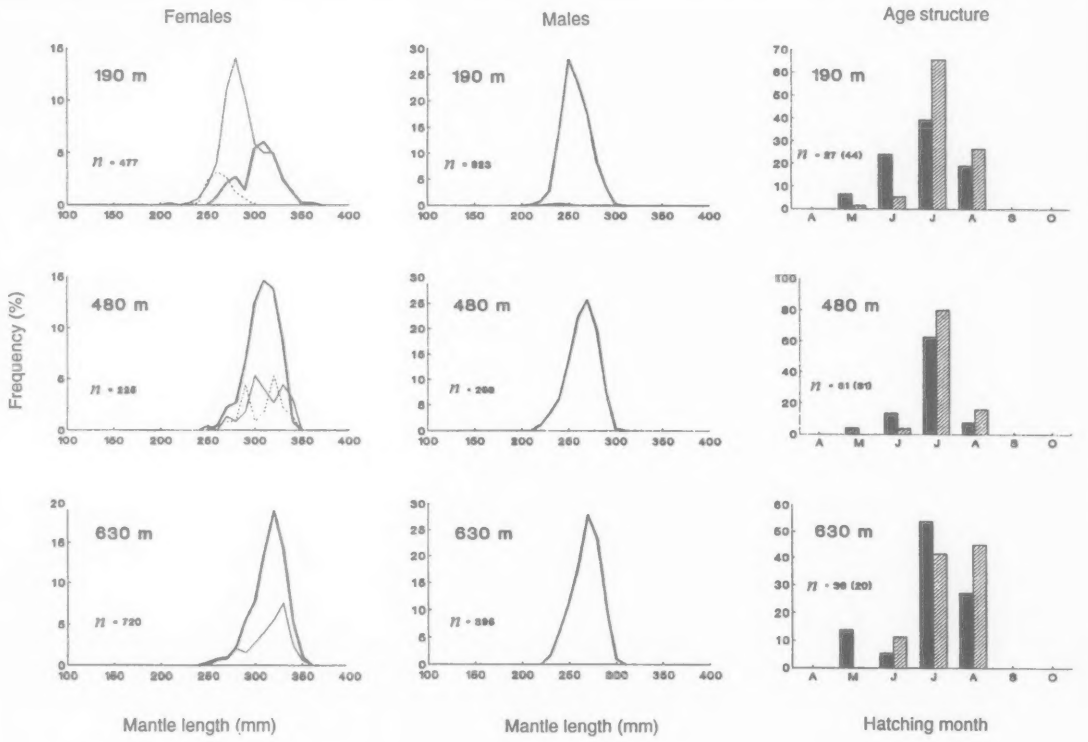

Figure 6

Length-frequency and hatching month compositions (age structures) of *Illex argentinus* at different depths of the fishery region of 45–47°S outside the EEZA between 21 and 30 April 1991. Symbols are the same as in Figure 4.

hatching-month compositions were practically similar to those for the continental slope (630 m) at 45–47°S and shelf (190–210 m) of 51–52°S. However, in spite of the similarity in modal length both in males and females in the last case, most of the females were mature in 45–47°S, whereas those at 51–52°S were still maturing (Figs. 5 and 8).

Discussion

Stock structure dynamics

Studies of the length-at-age structures for immature, maturing, and mature squid (separately) of both sexes, with a 10-day interval, revealed in detail the intrapopulation structure dynamics and migratory patterns of *I. argentinus* during the January–April feeding period on the Patagonian Shelf. Previous investigations, in which length-at-age data were pooled separately for each sex, revealed only general patterns in the age structure dynamics of *I. argentinus* (Rodhouse and Hatfield, 1990; Uozumi and Shiba, 1993).

Stock structure of winter-hatched *I. argentinus* was rather stable during the feeding period (January–April). After massive immigration of June- and July-hatched squid into the region 45–47°S from the end of January through the beginning of February, probably from an area farther north on the Patagonian Shelf (Hatanaka, 1988; Parfeniuk et al., 1992), the age structure of squid remained rather stable until the middle of April. During each 10-day period, from four to five month classes were observed, similar to the number obtained from the jigging fishery data (Uozumi and Shiba, 1993). Predominance of monthly classes changed gradually from June-hatched squid in February to July-hatched squid in March–April.

A considerable portion of the June and July-hatched squid continued their southward feeding migrations and reached 47–49°S by the end of February, which

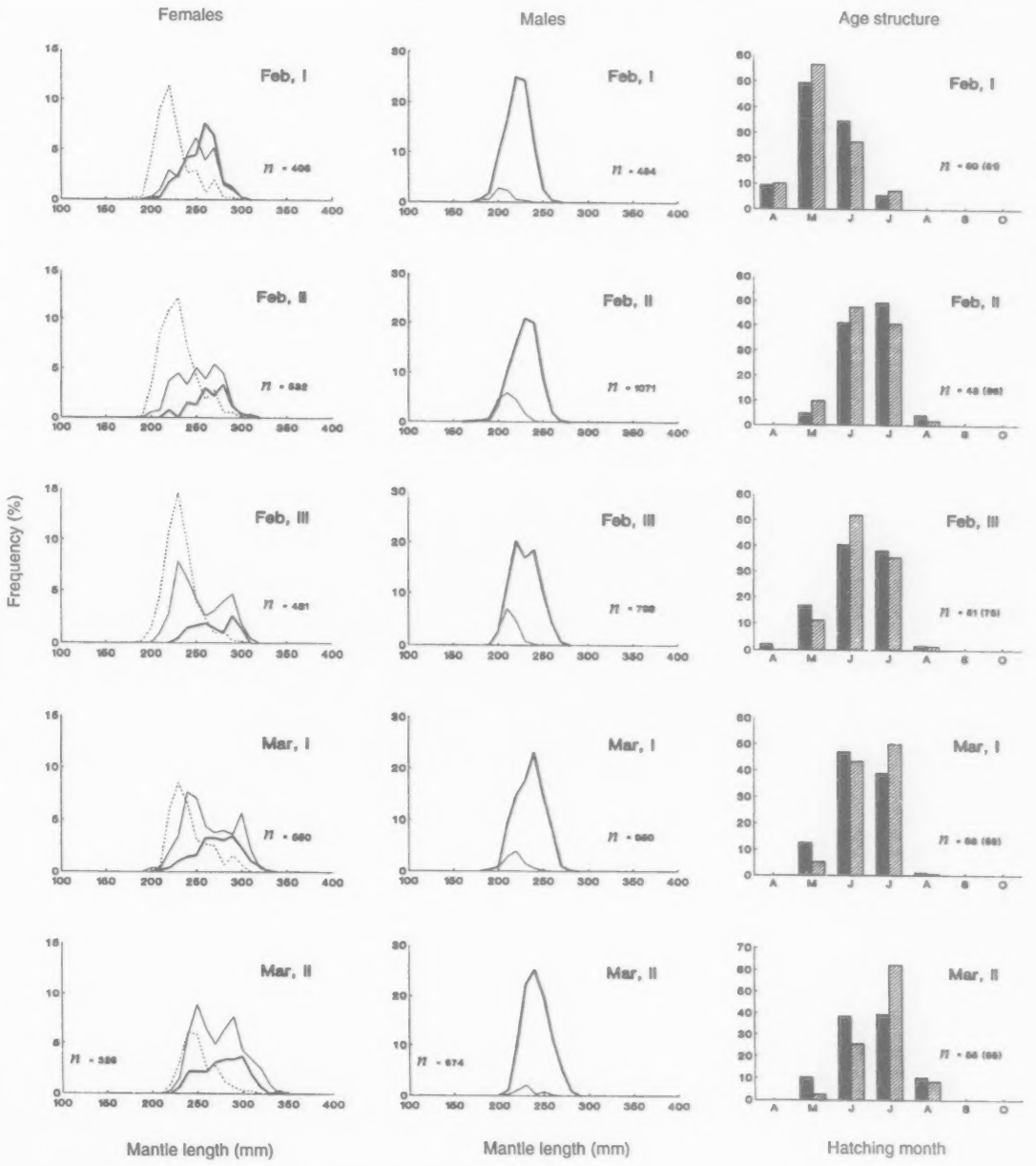

Figure 7

Length-frequency and hatching month compositions (age structures) of *Illex argentinus* of the fishery region of 47–49°S within the EEZA in February–March 1991. Symbols are the same as in Figure 4.

was confirmed both by similar hatching-month compositions and prominent peaks of the CPUE in both regions.

During the feeding period, winter-hatched *I. argentinus* grew rapidly and matured—males maturing at younger ages (from one to two months) than females

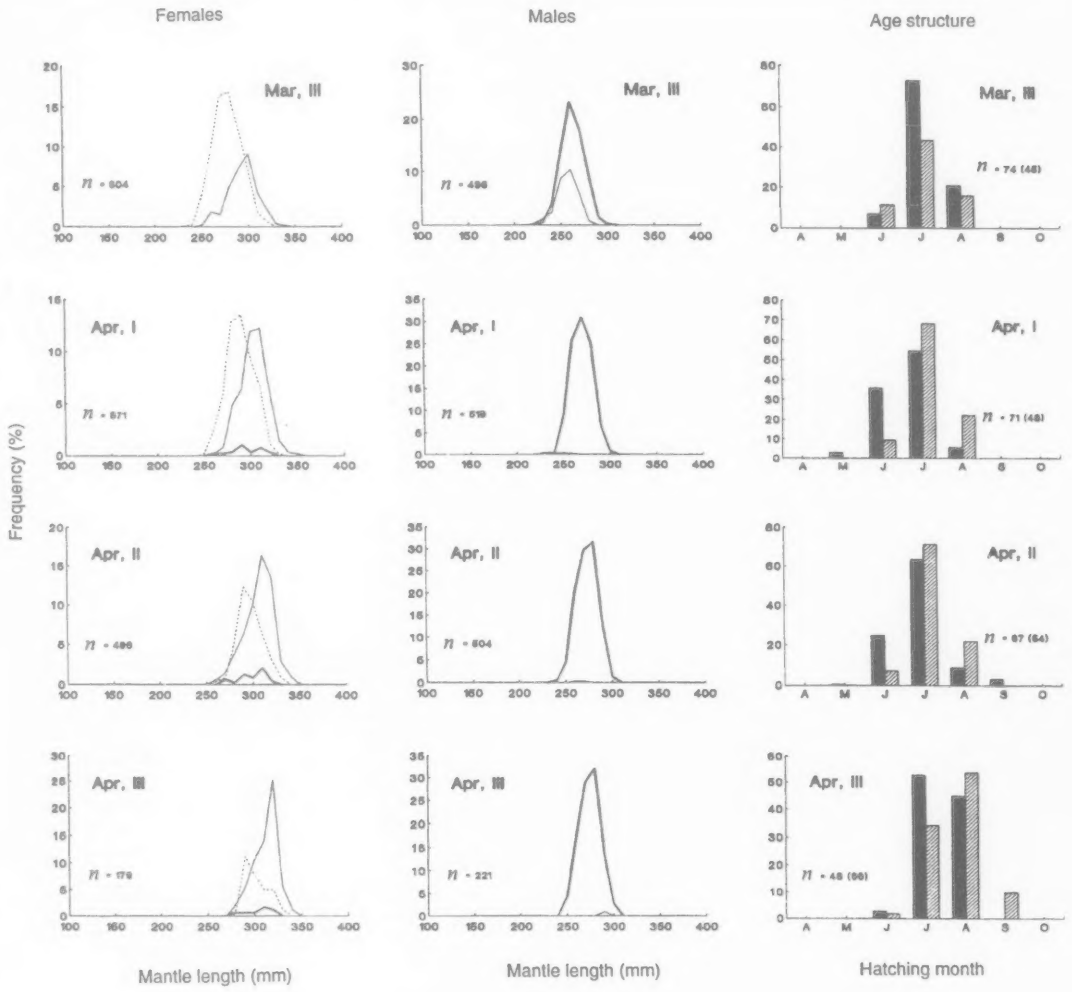

Figure 8

Length-frequency and hatching month compositions (age structures) of *Illex argentinus* of the fishery region of 51–52°S within the EEZA in March–April 1991. Symbols are the same as in Figure 4.

(Arkhipkin, 1990; Rodhouse and Hatfield, 1990). Taking into account the rather stable hatching month compositions during February–April, the squid were not performing any active spatial migrations after their arrival into the region of 45–49°S. Therefore, growth curves that were constructed on the basis of the increase in modal lengths of squid during February–April are valid, and growth rates calculated from these curves are probably close to actual growth rates (Koronkiewicz, 1986; Hatanaka, 1988). During feeding, some redistribution of the *I. argentinus* population was observed; large maturing and mature squid (mainly females) tended to shift from the shelf (130–150 m depth) in a northeast direction and to concentrate over the shelf edge (160–170 m depth). This shift resulted in an increase in their proportion in catches in the region outside the EEZA (45–47°S) and a simultaneous decrease in their proportion within the EEZA (47–49°S). A similar shift of maturing squid from the shelf to the shelf edge has been noted by Brunetti et al. (1998). Probably, this shift of maturing and mature females was also a reason for the consid-

erable predominance of males (2:1) in catches within the EEZA in February–March. Similar sex ratios have also been noted in the fishery region north of the Falkland Islands (Koronkiewicz, 1995). Another explanation for the predominance of males in shelf catches may be the earlier migrations of mature males (in contrast to females) from the southern part of their feeding area through the region of 47–49°S. This migratory pattern occurs during prespawning migrations of *I. argentinus* from the southern part of the Patagonian Shelf (Arkhipkin, 1993).

In April large mature males and females, which had been aggregated over the shelf edge, began to shift to the continental slope and migrate to great depths (>600 m), where they mixed with the already migrating schools of July- and August-hatched squid that had fed in the southern part of the Patagonian Shelf and around the Falkland Islands (Arkhipkin, 1993). Such a redistribution of *I. argentinus* aggregations caused a rather sharp decrease in the CPUE of jigging vessels on the shelf in April–May and a simultaneous regrouping of trawlers—a shift from the shelf to one over the continental slope (Hatanaka, 1988; Nigmatullin, 1989b). Medium-size squid remained on the shelf and probably made their prespawning migrations along the shelf edge.

Stock structure of winter-hatched *Illex argentinus*

From the complex of biological characteristics (statolith microstructure, modal length in different months, sizes at maturity, types of feeding, and prespawning migrations), it is possible to consider the Patagonian Shelf south of 45°S as a feeding ground for two intraspecific groups of winter-hatched *I. argentinus*: the "shelf group that matures at medium sizes" (ShG) and the "slope group that matures at large sizes" (SlG). These two groups correspond well with the bonaerensis north Patagonian stock (BNPS) and south Patagonian stock (SPS) distinguished by Brunetti (1988) by using length-frequency analysis. Later, Brunetti et al. (1998) postulated that the spawning of both groups takes place near the shelf edge and over the continental slope; the BNPS squid spawn north of 43°S in winter, whereas the SPS squid spawn south of 43°S in autumn. It was shown however that the SPS squid definitely migrated from the southern part of the Patagonian and Falkland shelves along the continental slope farther north at 41–42°S (Arkhipkin, 1993), but location of their spawning grounds is still unknown (Haimovici et al., 1998).

The shelf group also corresponds to the winter shelf group (WSG), and the slope group corresponds well to the winter oceanic group (WOG), both (WSG and WOG) of which were distinguished by different locations of juvenile feeding and by type of life cycle (Parfeniuk et al., 1992; Nigmatullin and Laptikhovsky, 1996).

The shelf group of *I. argentinus* has a neritic life cycle, characterized by the following features: spawning in warm waters of the northern part of the species range (27–36°S); southward feeding migrations of juveniles <100–150 mm ML over the Patagonian Shelf; a "shelf" type dark zone within the statolith microstructure (Arkhipkin, 1993); fast juvenile growth but rather slow growth of immature squid; medium sizes at maturation (males at 160–220 mm ML, females at 180–240 mm ML); medium maximum sizes for mature squid (males of 180–260 mm ML, females of 220–320 mm ML); and northward prespawning migrations over the shelf. The slope group of *I. argentinus* has an oceanic-slope life cycle characterized by the following features: slope spawning in the northern part of the species area (27–36°S); southward feeding migrations of juveniles <100–150 mm ML in the open part of the Argentine Basin; an "oceanic" type dark zone within the statolith microstructure (Arkhipkin, 1993); slow juvenile growth but rather fast growth of immature squid; large sizes at maturation (males at 180–240 mm ML, females at 240–340 mm ML); large maximum sizes for mature squid (240–340 mm ML, females up to 280–400 mm ML); and northward prespawning migrations over the continental slope. The taxonomic status of the two groups of winter-spawned *I. argentinus* remains unclear (Arkhipkin and Scherbich, 1991; Parfeniuk et al., 1992; Nigmatullin and Laptikhovsky, 1996; Santos and Haimovici, 1997).

Interannual changes in stock structure

It has been shown that growth rates of *I. argentinus* from the same hatching month vary to a lesser extent between different years from those of the different months of hatching within one year (Arkhipkin and Laptikhovsky, 1994). Thus it is possible to make comparisons of modal lengths of squid from the same month of hatching but in different years. The results of this study (based on data collected by the trawl fishery in 1991) are somewhat different from those obtained from the Japanese jigging fishery in 1989–1990 (Uozumi and Shiba, 1993). Generally, during the same month and in the same region of sampling, a majority of males and females caught by jigs in 1989 were about a month younger and correspondingly 20–30 mm smaller than those sampled by the trawl fishery in 1991 (Figs. 8 and 9 in Uozumi and Shiba, 1993; and Figs. 4 and 5 of the present study). Unfortunately, there are no data on the length-frequency composition of trawl-caught *I. argentinus* in 1989 (Arkhipkin and Laptikhovsky, 1994), and

thus it is difficult to explain the reasons for such a difference in length composition between the two years. It was found that *I. argentinus* caught by jigging gear were significantly larger and more mature (especially females) than trawl-caught squid fished in the same location and time (Koronkiewicz, 1995). Thus, differences in age and length compositions observed in 1989 and 1991 can be explained by interannual changes in population structure of *I. argentinus* rather than by various selectivity of the two different sampling gears.

The results of the present study show that in January–April, the international squid fishery in the southwest Atlantic catches aggregations of both groups of winter-spawned *I. argentinus*. Squid of the shelf group are captured by trawlers and jigging vessels over the depth range of 150–200 m mainly in the region of 45–49°S. Squid of the slope group are caught by trawlers and jigging vessels mainly in the southern part of the Patagonian Shelf within the EEZA (47–51°S) over the depth range of 150–250 m in February–March, and by trawlers over the continental slope (45–47°S) at depths of 600–700 m in April.

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Microsatellite DNA variation and estimation of stock composition of sockeye salmon, *Oncorhynchus nerka*, in Barkley Sound, British Columbia

Terry D. Beacham
Khai D. Le
Monique R. Raap
Kim Hyatt
Wilf Luedke
Ruth E. Withler

Pacific Biological Station
Department of Fisheries and Oceans
Nanaimo, British Columbia
Canada V9R 5K6

E-mail address (for T.D. Beacham): beachamt@pac.dfo-mpo.gc.ca

Abstract.—Microsatellite DNA variation at six microsatellite loci (Omy77, Ots3, Ots100, Ots103, Ots107, and Ots108) was examined in approximately 900 sockeye salmon, *Oncorhynchus nerka*, collected between 1987 and 1995 from three stocks on the west coast of Vancouver Island, British Columbia, Canada. Variation in allele frequencies among stocks was, on average, about 12 times greater than temporal variation within stocks. Individual locus F_{ST} estimates ranged from 0.013 to 0.107 among stocks, with an overall value of 0.056. Analysis of simulated mixed-stock samples indicated that data from four to six of the microsatellite loci surveyed would enable relatively accurate and precise estimates of stock composition for mixtures composed of fish from the three stocks. Application of the mixture analysis to 1100 fish sampled in Barkley Sound and Alberni Inlet fisheries during 1997 indicated that sockeye salmon from Great Central Lake constituted about 70% of the commercial catch. The later time of return of sockeye salmon from Henderson Lake than of those from Great Central or Sproat Lake as previously indicated by analysis of parasite frequencies was confirmed in the 1997 fishery sampling. Stock composition of catches varied among gears, presumably owing to gear selectivity.

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In the sockeye salmon (*Oncorhynchus nerka*) fishery in Barkley Sound on the west coast of Vancouver Island, three stocks (Sproat Lake, Great Central Lake, and Henderson Lake) account for all of the catch in the mixed-stock fishery (Hyatt and Steer, 1987) (Fig. 1). These stocks have been exploited for over 100 years, but the area of the fishery has changed. The present fishery is conducted over a wide area in Barkley Sound. Lake fertilization has been used to increase production of Barkley Sound sockeye salmon (LeBrasseur et al., 1978; Hyatt and Stockner, 1985). Of the lakes sampled in our study, Great Central Lake has been fertilized most extensively, with annual applications of fertilizer between 1970 and 1973, and from 1977 to the present. Sproat Lake was fertilized between 1985 and 1987, and Henderson Lake has been fertilized from 1976 to the present.

Assessment of the effects of fertilization on the productivity of Great Central and Henderson lakes required accurate and reasonably precise estimates of stock composition in the Barkley Sound sockeye salmon catch. The frequency of occurrence of two myxosporean parasites, *Myxobolus arcticus* in the brain and *Henneguya salmonicola* in the muscle, differed substantially among sockeye salmon in the three lakes during 1977–84 (Quinn et al., 1987), and these differences in prevalence were used to provide estimates of stock composition in the fishery until 1984 (Steer et al., 1986, 1988). Sockeye salmon from Sproat Lake and Great Central Lake accounted for 95% of the catch from 1980 to 1984 (Hyatt and Steer, 1987). In the 1990s, it became apparent that the frequency of occurrence of the two parasites had changed in Great Central Lake sockeye salmon (Beacham et al., 1998), and fishery managers no longer considered estimates of stock composition derived from parasites to be reliable for management decisions. The timing of the change in parasite frequency of occurrence between 1984 and the 1990s was unknown, rendering post-1984 estimates of stock composition and associated estimates of individual lake productivity uncertain. It became imperative to develop a reliable alternative method of stock identification that could be applied to fishery samples for accurate estimation of both catch and productivity by stock.

A preliminary survey of DNA variation at microsatellite loci indicated that there was some differentiation among the Barkley Sound sockeye salmon stocks (Nelson et al., 1998). Evaluation of alternative methods of stock identification indicated that mixture analysis based on microsatellite allele frequencies would likely provide reliable estimates of stock composition (Beacham et al., 1998). In the present study, we expanded the analysis of variation at microsatellite loci of Barkley Sound sockeye salmon to six polymorphic loci, examined the differentiation among and within stocks at each locus, evaluated the precision of data and accuracy of stock composition estimates for a range of mixture sample sizes based on data from three to six loci, and finally used the microsatellite variation to estimate stock compositions from 1997 fishery samples.

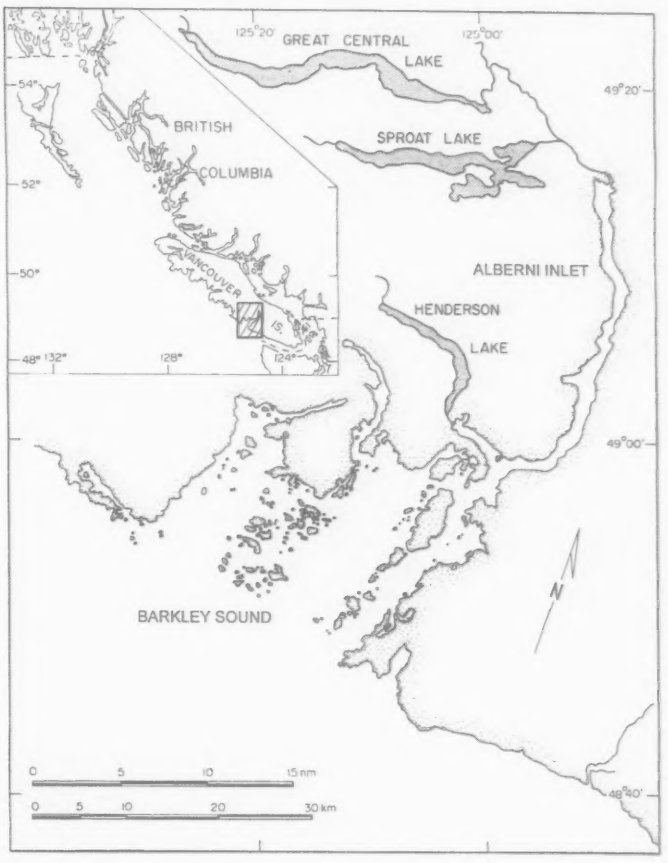

Figure 1

Location of Barkley Sound on Vancouver Island. Sockeye salmon are produced in Great Central Lake and Sproat Lake, both part of the Somass River drainage, as well in Henderson Lake.

Materials and methods

Collection of DNA samples and amplification by PCR

Scales were collected from sockeye salmon returning to spawn in the Sproat Lake and Great Central Lake drainages in 1987, 1990, and 1992. Scales were collected from Henderson Lake sockeye salmon in 1988 and 1993, and liver samples preserved in 95% ethanol were collected in 1995. Scales or operculum punches were collected from sockeye salmon sampled in fisheries in 1997. DNA was extracted from scales as outlined by Nelson et al. (1998). For the operculum or liver samples, approximately 0.3 g of tissue was placed in each well of a 96-well plate containing 0.2 mL of 5% chelex in TE buffer (10 mM Tris pH 7.4, 1 mM EDTA pH 8.0, 0.10 mg/mL proteinase K, and 0.1% SDS) and incubated for 15 min at 50°C, and then incubated for an additional 15 min at 95°C. The supernatant from each well was collected and placed in a fresh 96-well plate and stored at –20°C. About 1 mL of this extract was required for each amplification of the sample by the polymerase chain reaction (PCR).

Loci amplified by PCR were the dinucleotide repeats Omy77 and Ots3 and the tetranucleotide repeats Ots100, Ots103, Ots107, and Ots108 (Table 1). For all primer sets used in this study, PCR was conducted in 25-μL reactions containing 12 pmol (0.48 μM) of each primer, 80 μM of each nucleotide, 20 mM Tris-pH 8.8, 2 mM $MgSO_4$, 10 mM KCl, 0.1% Triton X-100, 10 mM $(NH_4)SO_4$, and 0.1 mg/mL of nuclease-free bovine serum albumin. Each PCR reaction was preceded by an initial denaturation step of three min at 94°C. All cycle extension (30 cycles for all loci except Ots108 which was 35 cycles) steps were for 60 sec at 72°C and all cycle denaturation steps were for 20 sec at 94°C. PCR of Omy77, Ots3, Ots100, Ots103, Ots107, and Ots108 was accomplished with annealing temperatures of 48°C, 50°C, 57°C, 55°C, 48°C, and 46°C, respectively. Annealing times were 30 sec for Omy77 and Ots100, and 60 sec for the other loci.

Gel electrophoresis and band analysis

PCR products were size fractionated on 16 cm × 17 cm nondenaturing polyacrylamide gels and visualized by

staining with 0.5 mg/mL ethidium bromide in water and ultraviolet light illumination. Nelson et al. (1998) provide a complete description of gel electrophoretic conditions. All gels were run for 14–18 h at 65–70 V, using 8% acrylamide for analysis of Ots100 and Ots103, and 10% acrylamide for analysis of Omy77, Ots3, Ots107 and Ots108. Twenty-nine lanes per gel were loaded. One outside lane contained a one-kb ladder (Gibco BRL), three lanes contained a 20-bp ladder (Gensura Labs Inc., Del Mar, CA) evenly spaced across the gel, one lane contained a standard fish to determine precision of estimation of allele size, and 24 lanes contained an individual fish for analysis.

Gels were scanned at a 1024 × 1024 pixel density with a Kodak charge coupled device (CCD) camera with low-light capability and a yellow filter. Images were analyzed by using BioImage Whole Band software (Genomic Solutions Inc., 1995), where the size of the amplified microsatellite alleles were reported to the nearest base pair (bp) based upon the molecular size grid created with the 20-bp markers.

Because some uncertainty occurred in estimation of allele size from the 20-bp grid, we identified alleles on the basis of a binning procedure (Gill et al., 1990). Peaks in the allele frequencies used to identify main alleles and bin widths generally corresponding to a repeat unit were set so that the main allele was located in the middle of the bin. Precision of estimation of allele size was evaluated with the standard fish analyzed for each locus.

Data analysis

Annual variation in allele frequencies within populations was tested with GENEPOP version 3.1 with the Markov-Chain approach by using χ^2 probability values (Raymond and Rousset, 1995). The dememorization number was set at 1000, and 50 batches were run for each test with 1000 iterations/batch (Raymond and Rousset, 1995). Each stock at each locus was tested for departure from Hardy-Weinberg equilibrium by using GENEPOP. Gametic linkage disequilibrium between loci in each population was also evaluated with GENEPOP. Tests of genetic differentiation with three pairwise comparisons among the populations were also conducted with GENEPOP with the Markov-Chain approach by using χ^2 probability values. Critical significance levels for simultaneous tests were evaluated by using sequential Bonferroni adjustment (Rice, 1989). F_{ST} estimates for each locus were calculated with GENEPOP, and the standard deviation of the estimate for an individual locus was determined with FSTAT (Goudet, 1995) by jackknifing over stocks and for all loci combined by bootstrapping over loci. Estimation of variance components of stock differences and annual variation within stocks was determined with BIOSYS (Swofford and Selander, 1981). Principal components of nine (three annual samples multiplied by three stocks) composite arrays of allele frequencies for six loci were calculated with the PRINCOMP procedure in SAS (SAS, 1989).

Estimation of stock composition

The effectiveness of using variation at microsatellite loci for the practical assessment of stock composition in mixed-stock fisheries of Barkley Sound was evaluated from the stand points of precision of stock composition data and accuracy of stock composition estimates in simulated fishery samples. Although only three stocks could contribute to the fishery samples, we wished to determine the sample size required to detect accurately the relatively small proportion of Henderson Lake sockeye salmon that were expected to be present in most fishery samples. In addition, we wished to examine the effect of the number of loci used in the estimation of stock composition. The simulated mixtures were composed of 30% Sproat Lake fish, 60% Great Central Lake fish, and 10% Henderson Lake fish because these proportions are the approximate long-term mean of the Barkley Sound fishery.

Allele frequencies were determined for each locus in each stock, and the model of Fournier et al. (1984) was used to estimate stock composition by the condi-

Table 1

Primer sequences for the microsatellite loci analyzed in the study.

Locus	Sequence (5'–3')	Source
Omy77	F: CGT TCT CTA CTG AGT CAT R: GGG TCT TTA AGG CTT CAC TGC A	Morris et al. (1996)
Ots3	F: CAC ACT CTT TCA GGA G R: AGA ATC ACA ATG GAA G	Banks et al. (1999)
Ots100	F: TGA ACA TGA GCT GTG TGA G R: ACG GAC GTG CCA GTG AG	Nelson et al. (1998)
Ots103	F: AGG CTC TGG GTC CGT G R: TGA TAT GGT GTG ATA GCT GG	Beacham et al. (1998)
Ots107	F: ACA GAC CAG ACC TCA ACA R: ATA GAG ACC TGA ATC GGT A	Nelson and Beacham (1999)
Ots108	F: TCT GTT TAT CTT TCT ATT A R: AAG GAG AGA CAG AGG G	Nelson and Beacham (1999)

tional maximum likelihood method. Baseline genotypic frequencies for each of the three stocks were calculated from the observed allele frequencies under the assumption of Hardy-Weinberg equilibrium. Each baseline stock was resampled with replacement in order to simulate random variation involved in the collection of the baseline samples during the estimation of stock composition of each mixture. Hypothetical fishery samples of 100–300 fish with fixed stock composition were generated by randomly resampling with replacement the baseline stocks, and adding the appropriate number of fish from each stock to the mixture. Estimated stock composition of the mixture was then determined, and the whole process was repeated 100 times to estimate the mean and standard deviation of the individual stock composition estimates.

Table 2

Precision of estimates of allele size (in basepairs) at each microsatellite locus for standard fish run only once per electrophoretic gel. *n* is the number of gels on which allele sizes for a standard fish were estimated. Standard deviation is given in parentheses.

Locus	*n*	Allele size	Range	Allele size	Range
Ots3	15	74.1 (0.35)	74–75	93.1 (0.35)	93–94
Omy77	28	94.9 (0.63)	94–96	110.3 (0.53)	109–111
	24	100.4 (0.53)	100–101	116.0 (0.62)	115–117
	8	104.0 (0.00)	104–104	116.1 (0.64)	115–117
Ots107	46	109.7 (0.55)	109–111	117.7 (0.48)	117–118
Ots108	12	112.1 (0.67)	111–113	184.6 (0.51)	184–185
Ots100	8	158.3 (0.71)	157–159	184.4 (1.30)	183–186
	26	164.5 (0.71)	163–166	181.6 (0.64)	180–183
	11	158.4 (0.50)	158–159	196.5 (0.52)	196–197
Ots103	33	175.1 (0.60)	174–176	213.4 (1.00)	211–215

Fishery samples

In 1997, samples were collected from three commercial gillnet fishery openings in Barkley Sound, a gillnet test fishery, a purse-seine test fishery, the recreational fishery, and an aboriginal fishery. The commercial gillnet fishery was conducted primarily in Barkley Sound, with gillnet mesh sizes ranging from 114 mm (4.5 inches) to 133 mm (5.25 inches). The gillnet test fishery was conducted farther inland at the head of Barkley Sound and at the mouth of Alberni Inlet with a gill net 110 m (60 fathoms) in length and 180 meshes deep, and having a mesh size of 114 mm. Samples from the purse-seine fishery, the recreational fishery, and the aboriginal fishery were derived entirely from Alberni Inlet. The recreational fishery was conducted near the head of Alberni Inlet and the aboriginal fishery, conducted at the head of Alberni Inlet and in the Somass River, was the most terminal fishery. Estimated stock contributions to each sample were determined as a point estimate from all the fish in the sample, and standard deviations of the estimates were derived from bootstrap resampling of both the baseline stocks and the mixture.

Results

Precision of estimation of allele size

Standard deviations of the estimated allele sizes for the heterozygous standard fish analyzed at each locus ranged from 0.00 to 1.30 and tended to increase with allele size (Table 2). For both alleles at Ots3, 100% of the estimated sizes for each allele spanned a 2-bp interval. For alleles <110 bp at Omy77, 93% (56/60) of the estimated sizes of the allele were in a 2-bp interval, as were 90% of the estimated sizes of alleles between 110 and 120 bp. Estimated sizes of alleles of the standard fish that were analyzed at the other loci were all estimated within a 4-bp interval for alleles <200 bp, with 85% of the estimated sizes of the larger allele (213 bp) at Ots103 within a 4-bp interval.

Variation within stocks

All six microsatellite loci examined were polymorphic for all three stocks. Observed heterozygosity of the loci examined over all stocks was as follows: Omy77 0.70 (stock range 0.61–0.80), Ots3 0.67 (0.64–0.70), Ots100 0.75 (0.69–0.79), Ots103 0.83 (0.81–0.86), Ots107 0.28 (0.17–0.40), and Ots108 0.85 (0.80–0.89). Significant departures (correction for three tests per locus, α=0.0167) from the expected Hardy-Weinberg distribution of genotypic frequencies were observed at the Omy77 locus in all stocks, owing, in the case of Sproat and Henderson lakes, to a deficiency of heterozygotes. A similar significant heterozygote deficiency was also detected at Ots108 in Sproat Lake sockeye salmon. Significant annual variation (correction for six tests per stock, α=0.0083) in allele frequencies was observed at Omy77 in Sproat Lake and Henderson Lake sockeye salmon, and at Ots108 in Henderson Lake sockeye salmon. No significant linkage disequilibrium between any pair of loci in any stock was observed.

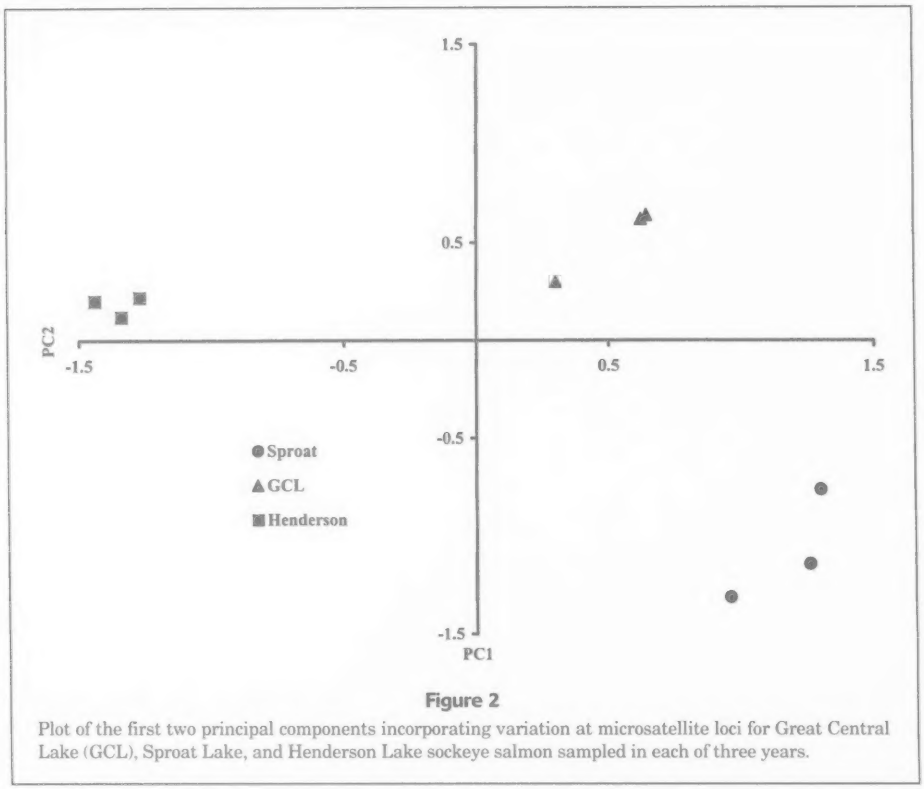

Figure 2

Plot of the first two principal components incorporating variation at microsatellite loci for Great Central Lake (GCL), Sproat Lake, and Henderson Lake sockeye salmon sampled in each of three years.

Variation among stocks

The three sockeye salmon stocks in Barkley Sound were genetically distinct at all six loci examined. All pairwise tests of allele frequencies among stocks were highly significant at all loci ($P<0.001$). At Omy77, the frequency of the Omy77^{94} allele ranged from 0.005 in Henderson Lake sockeye salmon to 0.449 in Sproat Lake fish, and the frequency of Omy77^{104} ranged from 0.216 in Sproat Lake fish to 0.546 in Henderson Lake fish (Table 3). Substantial differentiation in allelic frequencies among stocks was observed at Ots3. For example, the frequency of Ots3^{88} in Henderson Lake sockeye salmon was 0.114, whereas in Sproat Lake fish it was 0.543. Similarly, the frequency of Ots3^{99} was 0.059 in Sproat Lake fish and 0.243 in Henderson Lake fish. At Ots100, the frequency of Ots100^{158} ranged from 0.256 in Sproat Lake sockeye salmon to 0.504 in Henderson Lake fish (Table 3). Although the three stocks were distinct at Ots103, the allele frequency variation was less marked at that locus. At Ots107, the combined frequency of four alleles (81, 109, 113, 117) was greater than 0.95 in all stocks, but stock differentiation was nonetheless apparent. For example, the frequency of Ots107^{81} was 0.135 in Great Central Lake sockeye salmon, but < 0.010 in the other two stocks (Table 3). Variation in allelic frequencies at Ots108 was evident among stocks, with the frequency of Ots108^{122} ranging from 0.000 to 0.196. Strong genetic differentiation among these three stocks was evident at all six microsatellite loci.

Comparison of the relative magnitude of differentiation among stocks and among samples from the same stock collected in different years showed that differentiation among stocks always exceeded temporal variation within stocks and was on average 12 times greater (Fig. 2; Table 4). At Ots3, the differences among stocks were 235 times greater than the observed annual variability. With data combined over years, individual locus F_{ST} estimates ranged from 0.013 to 0.107, with an overall value of 0.056 (Table 4). The loci displaying the greatest differentiation among stocks were Ots3 and Omy77, whereas Ots103 displayed the least differentiation. Sproat Lake and Great Central Lake stocks, both in the same river drainage, were genetically the most similar (pairwise F_{ST} estimate over all loci: 0.032). The Great Central Lake and Henderson Lake stocks were more genetically distinct

Table 3

Observed allele frequencies at six microsatellite loci for three stocks of Barkley Sound sockeye salmon. Alleles have been designated by the lower size limit of the bin. *n* is the number of fish scored at each locus in each stock.

Allele	Sproat	Great Central	Henderson	Allele	Sproat	Great Central	Henderson
Omy7				Ots103			
n	264	303	305	*n*	221	304	308
90	0.000	0.000	0.002	144	0.007	0.021	0.000
92	0.011	0.003	0.002	152	0.043	0.026	0.000
94	0.449	0.172	0.005	156	0.027	0.002	0.002
96	0.042	0.020	0.000	160	0.009	0.018	0.003
98	0.006	0.007	0.000	164	0.034	0.008	0.013
100	0.125	0.231	0.277	168	0.016	0.015	0.006
102	0.011	0.015	0.030	172	0.038	0.033	0.019
104	0.216	0.361	0.546	176	0.020	0.025	0.034
106	0.002	0.002	0.020	180	0.029	0.064	0.080
108	0.040	0.005	0.000	184	0.032	0.076	0.057
110	0.051	0.054	0.100	188	0.050	0.178	0.073
112	0.002	0.003	0.002	192	0.305	0.268	0.312
114	0.004	0.086	0.008	196	0.269	0.151	0.185
116	0.038	0.040	0.010	200	0.075	0.079	0.130
118	0.004	0.002	0.000	204	0.043	0.028	0.071
Ots3				208	0.002	0.008	0.011
n	219	296	303	212	0.000	0.000	0.003
74	0.112	0.127	0.086	Ots107			
78	0.000	0.000	0.003	*n*	269	307	310
80	0.000	0.000	0.002	81	0.006	0.135	0.000
82	0.000	0.003	0.005	101	0.000	0.002	0.000
84	0.002	0.000	0.000	105	0.000	0.000	0.005
86	0.000	0.002	0.000	109	0.084	0.062	0.053
88	0.543	0.378	0.114	113	0.866	0.762	0.913
92	0.002	0.007	0.000	117	0.030	0.036	0.029
93	0.274	0.394	0.526	121	0.015	0.003	0.000
96	0.000	0.002	0.005	Ots108			
97	0.007	0.005	0.013	*n*	214	199	269
99	0.059	0.073	0.243	122	0.196	0.106	0.000
103	0.000	0.005	0.000	126	0.014	0.035	0.004
105	0.000	0.005	0.003	130	0.002	0.000	0.000
Ots100				133	0.002	0.000	0.000
n	242	321	282	137	0.000	0.128	0.002
130	0.010	0.003	0.000	141	0.002	0.008	0.002
134	0.039	0.006	0.000	145	0.007	0.010	0.000
138	0.000	0.002	0.000	149	0.121	0.038	0.035
142	0.006	0.000	0.002	153	0.056	0.098	0.007
150	0.008	0.002	0.004	156	0.136	0.095	0.178
154	0.025	0.037	0.025	160	0.086	0.146	0.229
158	0.256	0.364	0.504	164	0.189	0.163	0.158
162	0.169	0.064	0.133	168	0.042	0.070	0.048
166	0.052	0.047	0.064	172	0.035	0.038	0.043
170	0.002	0.003	0.007	177	0.068	0.050	0.216
174	0.002	0.006	0.004	182	0.016	0.010	0.039
179	0.324	0.202	0.193	187	0.019	0.005	0.035
184	0.085	0.115	0.050	192	0.005	0.000	0.004
190	0.008	0.107	0.007	197	0.002	0.000	0.000
195	0.012	0.039	0.009				
200	0.000	0.002	0.000				

(pairwise F_{ST} estimate: 0.042), and the Sproat Lake and Henderson Lake stocks showed the greatest differentiation (pairwise F_{ST} estimate: 0.091).

Estimation of stock composition

The three loci with the highest F_{ST} estimates (Omy77, Ots3, and Ots107) also possessed the highest ratio of spatial to temporal variation (Table 4) and were therefore selected to form the core database for the analysis of the simulated mixtures. The number of loci used in the determination of stock composition or mixture sample size had little effect upon the accuracy of the estimated stock compositions (Table 5). Precision of the estimated stock compositions increased as both the number of loci and mixture size used in the determination increased. However, different options were available to obtain estimates of a desired precision. For example, higher levels of precision were obtained with four loci (Omy77, Ots3, Ots107, Ots100) in conjunction with a 150-fish sample size (600 units of data) than with all six loci and a 100-fish sample (600 units of data) (Table 5). The coefficient of variation for the estimated proportion of the predominant Great Central Lake stock was always less than that for estimated proportions of the other two stocks in the mixture. For the 4 loci in 150-fish mixture analysis, the coefficient of variation for the estimated proportion of Great Central Lake fish was 12%, whereas it was 22% for Sproat Lake fish, and 38% for Henderson Lake fish. The simulations indicated that fewer than six microsatellite loci could be used to provide reasonably precise data and accurate estimates of sockeye salmon stock composition for Barkley Sound fishery samples.

Conditional maximum likelihood estimation can overestimate the relative abundance of rare stocks. For Barkley Sound, this would likely be the Henderson Lake stock. The precision of data and accuracy of stock compositions estimates were investigated for mixture samples of 100 fish composed of 2% Henderson Lake (38% Sproat Lake, 60% Great Central Lake) and 5% Henderson Lake (35% Sproat Lake, 60% Great Central), where stock compositions were estimated by using the four microsatellite loci (Omy77, Ots3, Ots100, and Ots107) generally used for estimation of stock compositions in the 1997 fishery samples. Estimated stock compositions of the simulated 100 mixtures for the 2% Henderson Lake composition were 2.5% (SD=3.1%) Henderson Lake, 34.3% (SD=9.3%) Sproat Lake, and 60.4% (SD=9.3%) Great Central Lake. For the 5% Hender-

Table 4

F_{ST} estimates and the ratio of the variance components attributable to among and within stock differentiation (over time) for six microsatellite loci of Barkley Sound sockeye salmon. Standard deviation of F_{ST} estimates is given in parentheses.

Locus	F_{ST}	Variance ratio
Omy77	0.107 (0.094)	20.3
Ots3	0.099 (0.089)	235.2
Ots100	0.027 (0.013)	5.8
Ots103	0.013 (0.007)	3.9
Ots107	0.043 (0.035)	6.3
Ots108	0.039 (0.018)	3.6
All	0.056 (0.032)	11.8

Table 5

Average estimated stock composition (%) of simulated mixtures of Barkley Sound sockeye salmon based on variation at three to six microsatellite loci. True mixture percentages were as follows: Sproat Lake 30%, Great Central Lake 60%, and Henderson Lake 10%. The three loci initially used to estimate the percentages of the mixtures were Omy77, Ots3, and Ots107, and Ots100, Ots108, and Ots103 were added sequentially. Each mixture was generated 100 times with replacement, and stock compositions of the mixtures were estimated by resampling each baseline stock with replacement to obtain a new distribution of allele frequencies, with the same sample size in the new distribution as in the original one. Standard deviation is given in parentheses.

Source and loci	Mixture size (no. of fish)			
	100	150	200	300
Sproat Lake				
3	30.7 (9.98)	31.1 (8.51)	30.8 (6.81)	30.7 (6.48)
4	30.7 (8.21)	30.8 (6.87)	30.8 (6.38)	30.2 (4.44)
5	30.1 (7.54)	30.6 (5.87)	30.0 (4.44)	29.4 (4.18)
6	31.2 (7.23)	29.0 (5.27)	29.8 (4.42)	29.8 (4.00)
Great Central Lake				
3	60.5 (11.74)	60.1 (9.27)	60.2 (7.40)	59.7 (7.74)
4	60.4 (9.41)	59.8 (7.40)	59.7 (6.88)	60.7 (5.21)
5	60.1 (8.26)	60.0 (6.63)	60.1 (5.11)	60.8 (4.50)
6	58.8 (8.03)	60.9 (5.96)	60.1 (5.09)	59.9 (4.31)
Henderson Lake				
3	8.8 (5.62)	8.8 (4.84)	9.0 (3.89)	9.6 (3.98)
4	8.8 (5.30)	9.3 (3.53)	9.6 (3.52)	9.1 (2.89)
5	9.8 (4.56)	9.5 (3.73)	9.9 (2.76)	9.8 (2.52)
6	10.0 (4.33)	10.1 (3.70)	10.0 (2.81)	10.3 (2.39)

son Lake composition, estimated stock compositions were 5.3% (SD=3.9%) Henderson Lake, 34.3% (SD=8.2%) Sproat Lake, and 60.4% (SD=8.9%) Great Central Lake. No significant bias was observed when Henderson Lake sockeye salmon composed 5% or less of the mixture.

Table 6

Estimated stock compositions (%) for sockeye salmon from three lakes sampled in gillnet test fisheries, seine test fisheries, commercial fishery openings, a native fishery, and recreational fishery in Barkley Sound during 1997. Four loci (Ots3, Ots100, Ots107, and Omy77) were used to estimate stock composition. *n* is the number of fish analyzed, and standard deviation of the estimates is given in parentheses.

Source	Week ending	*n*	Sproat	Great Central	Henderson
Commercial	4 Jul	118	25.8 (7.3)	73.9 (8.2)	0.3 (2.8)
Commercial[1]	11 Jul	95	22.3 (6.2)	71.1 (7.9)	6.2 (3.8)
Commercial[1]	18 Jul	95	29.7 (6.9)	63.0 (7.8)	7.3 (4.8)
Seine	27 Jun	120	35.9 (7.3)	56.6 (8.0)	7.5 (3.4)
Seine	4 Jul	111	48.4 (8.6)	42.2 (10.4)	9.0 (5.4)
Seine	18 Jul	117	37.3 (7.0)	59.6 (7.8)	3.1 (4.6)
Gillnet	20 Jun	50	34.4 (10.3)	65.6 (10.4)	0.0 (0.9)
Gillnet	27 Jun	50	36.2 (10.9)	51.0 (12.9)	12.9 (6.8)
Gillnet	4 Jul	50	21.2 (9.2)	71.9 (10.5)	6.7 (5.1)
Gillnet	11 Jul	50	33.1 (9.7)	54.1 (11.7)	12.8 (7.7)
Gillnet	18 Jul	50	24.5 (11.8)	72.2 (13.6)	3.4 (6.3)
Gillnet	25 Jul	50	19.9 (11.5)	60.0 (13.8)	20.2 (9.1)
Gillnet	1 Aug	50	28.2 (11.6)	42.7 (13.7)	29.1 (10.3)
Aboriginal	18 Jul	86	45.0 (8.4)	52.8 (8.5)	2.2 (2.3)
Recreational	18 Jul	33	33.5 (12.3)	54.8 (13.3)	11.7 (8.4)

[1] Additional loci, Ots103 and Ots108, were used in estimation of stock composition.

Application of estimates to 1997 fisheries

Although estimated stock contributions varied according to sampling period, sockeye salmon from Great Central Lake tended to predominate in all fisheries at any week (Table 6). However, differences in stock composition estimates among fishing gears were evident. In the commercial gillnet fishery, Great Central Lake sockeye salmon constituted about 70% of the catch (Table 6). In the gillnet test fishery, the proportion of Great Central Lake sockeye generally varied between 55 and 75% prior to July 25th. In the purse-seine test fishery, they accounted for about 50–55% of the catch. Higher proportions of Great Central Lake sockeye salmon were observed in the selective gillnet gear than in the more nonselective purse-seine gear. For example, for the week ending 4 July, Great Central Lake sockeye were estimated to have represented 70–75% of the catch in the commercial gillnet fishery and in the gillnet test fishery, but only about 40% of the catch in the seine test fishery. Although the samples analyzed from the purse-seine fishery were derived from more inland locations than those from the commercial and test gillnet fisheries, the differences in proportions of Great Central sockeye salmon more likely resulted from differences in gear selectivity than from differences in stock distribution because fish from all three stocks are generally distributed throughout Barkley Sound and Alberni Inlet when present.

Sockeye salmon stock from Henderson Lake are the smallest salmon exploited in the fishery, and thus the most vulnerable to overfishing in the mixed-stock harvest that takes place. Henderson Lake fish, which do not have to travel through Alberni Inlet in their spawning migration, were apparently caught in fisheries throughout Alberni Inlet, although there was a high degree of uncertainty about whether they were caught in the aboriginal fishery at the extreme head of Alberni Inlet (Table 6). Henderson Lake sockeye salmon generally represent 10% or less of the catch, except for sockeye salmon sampled after 18 July in the gillnet test fishery, when the relative abundance of Henderson Lake sockeye salmon substantially increased. By late July, Henderson Lake sockeye salmon constituted nearly 30% of the gillnet test fishery sample.

Discussion

DNA variation at microsatellite loci is becoming an increasingly important tool in fisheries research and management (see review by O'Connell and Wright [1997]). In salmonids, microsatellite loci are generally characterized by high levels of variability and differentiation among spawning populations (Angers et al., 1995; McConnell et al., 1997; Seeb et al., 1998), even in very localized areas (Beacham and Dempson, 1998). The feasibility of applying biological markers to salmon stock identification is enhanced when they display limited annual variation. With temporal stability of the discriminating characters, annual surveys of contributing populations are unncecssary once they have been adequately characterized. As for other neutral genetic markers (Wood et al., 1994; Beacham et al., 1996), temporal stability of allele frequencies at microsatellite loci has generally been observed in salmonid populations (Small et al., 1998). For popula-

tions in which annual variation has been detected, the magnitude of variation has been substantially less than that among populations (Nielsen et al., 1997; Beacham and Wood, 1999).

For sockeye salmon, in which the greatest geographic determinant of neutral genetic differentiation is the nursery lake (Wood, 1995), the task of identifying the contributions of three different lake systems to a mixed-stock sample should be relatively straightforward. Although significant genetic variation can occur among spawning sockeye salmon subpopulations isolated by time or space (or both) within a lake system, the extent of this variation is consistently much less than that observed among lakes—even those lakes within a single drainage system (Wood, 1995). Each of the three lakes is the confluence of multiple tripu-taries and may harbor genetically differentiated subpopulations of sockeye salmon. The spawning ground samples in our study were collected from locations within each lake system at which fish from more than one subpopulation may have been present, and different subpopulations may have been sampled among years. Thus, the departure of Omy77 (and Ots108 for Henderson Lake) genotypes from Hardy-Weinberg equilibrium and significant annual variation observed at these loci might both have reflected subpopulation differentiation in allele frequencies. It is unlikely that the heterozygote deficiency observed at Omy77 in Sproat Lake and Henderson Lake sockeye salmon would be a result of a null allele because genotypic frequencies of other sockeye salmon stocks surveyed at this locus have been in Hardy-Weinberg equilibrium (Beacham and Wood, 1999). Nevertheless, the level of differentiation at Omy77 was about 20 times greater among lakes than was the temporal variation observed within lakes. For all six microsatellite loci surveyed, differences among lakes were on average 12 times greater than variation within populations, confirming the relative stability of the microsatellite loci in Barkley Sound sockeye salmon populations over the 5–8 yr sampling period.

The six microsatellite loci used in the current study were also surveyed in nine sockeye salmon stocks of the Nass River drainage in northern British Columbia (Beacham and Wood, 1999). In the Nass River, the three loci displaying the greatest differentiation among stocks were Ots100 (F_{ST}=0.131), Ots3 (F_{ST}=0.111), and Ots108 (F_{ST}=0.084), whereas in the Barkley Sound stocks, the three most discriminating loci were Omy77 (F_{ST}=0.107), Ots3 (F_{ST}=0.099), and Ots107 (F_{ST}=0.043). The fact that loci differed in their relative levels of variation between the two areas is not surprising given the rapid evolution of microsatellite loci and the likelihood that the regions were founded postglacially by different sockeye salmon "races" (Wood, 1995). For stock identification applications, surveys of microsatellite variation in each geographic region of interest will generally be necessary to determine which loci are the most effective in differentiating local populations.

Effective assessment and management of sockeye salmon production in Barkley Sound is dependent upon determination of stock composition in fishery catches. Previous evaluation has indicated that the application of microsatellite technology to stock identification can provide the most reliable and cost-effective results (Beacham et al., 1998), but determination of the feasibility of such technology for Barkley Sound fisheries awaited examination of the relation between the number of loci used, the sample size of the stock mixture to be analyzed, and the precision of the estimated stock contributions. For any stock identification application, the optimal combination of number of loci surveyed and number of fish sampled from the catch is dependent on the genetic distance among stocks, the desired precision for an individual stock estimate, and the cost of the analysis for each locus.

The simulated mixtures evaluated for Barkley Sound sockeye salmon indicated that microsatellite variation could be used to provide accurate and reasonably precise estimates of individual stocks in the catch mixtures. They further indicated that although genotypic frequencies at Omy77 and Ots108 were not in Hardy-Weinberg equilibrium in some stocks, but assumed to be so in the stock composition estimation procedure, the violation of this assumption did not have a marked influence on the accuracy of the estimated stock compositions. The precision, but not accuracy, of the estimated contributions increased with both the number of loci (from 3 to 6) and the sample size of the mixture (from 100 to 300). For sample sizes of 150 fish and larger, a greater increase in precision for stock contribution estimates could always be achieved by increasing the number of loci surveyed to six than by increasing the sample size to 300. However, these simulations did not include estimation of the random error associated with sampling only a portion of the catch, and this error will always be reduced by increasing sample size. The level of precision of an estimated stock contribution increased with the contribution of the stock to the mixture. For estimation of the more abundant Great Central and Sproat sockeye salmon, the increase in precision afforded by additional data was approximately equivalent whether more fish (beyond 150) or more loci were analyzed (i.e. approximately equally precise stock contribution estimates were achieved by analyzing four loci in 300 fish and six loci in 200 fish). However, estimation of the small (10%) Henderson Lake contribution to the mixture was more sensitive to sample size and was more precise in the analysis of four loci in 200 fish than of six loci in 150 fish.

Successful application of microsatellite loci to estimation of stock composition in mixed-stock fisheries requires that loci be chosen that highlight differences among stocks to be separated and that adequate numbers of fish in the baseline stocks be surveyed to provide reliable estimates of allele frequencies, and thus genotypic frequencies used in the conditional maximum likelihood analysis. Microsatellite loci can contain a large number of alleles, and baseline sample sizes need to be of sufficient size to ensure that alleles present in fish from a stock in the mixture have also been observed in the baseline samples. Binning low-frequency similar-size alleles (Small et al., 1998) is also a strategy to consider in practical applications.

Although simulated mixtures can provide insights into the expected performance of the mixture analysis, the stock contribution estimates for actual fishery samples can only be evaluated by corroboration with data from other sources. Two supportive sources of independent information occur: time of return of the Henderson Lake stock and the typical catch composition for Barkley Sound that was previously derived from parasites. In Barkley Sound, the time of return of Henderson Lake sockeye salmon has been reported to be later than that of either Sproat Lake or Great Central Lake fish (Steer et al., 1988). For example, in 1984, Henderson Lake sockeye salmon were evident, on the basis of parasite analysis, in the commercial fishery prior to 27 June but increased in relative abundance after that time. The current analysis indicated that Henderson Lake sockeye salmon were absent from, or at low abundance in, the 1997 commercial fishery prior to the week of 4 July. Analysis of the gillnet test fishery and purse-seine samples indicated that the proportion of Henderson Lake sockeye salmon in those catches was low until mid-July but thereafter was substantial, consistent with a later time of arrival of the Henderson stock in Barkley Sound. In a typical return year, about 60% of the Barkley Sound sockeye salmon catch is derived from Great Central Lake, 30% from Sproat Lake, and 10% from Henderson Lake (Steer et al., 1988). Estimated stock compositions for the 1997 fishery catches are in reasonable agreement with the expected stock contributions. Results of the simulation analysis indicated that more precise, but not necessarily more accurate, estimates of the stock contributions (especially that from Henderson Lake) could have been obtained for the fishery catches if sample sizes had been larger than 50 (for the gillnet test fisheries) or approximately 100 (for the purse-seine test and commercial gillnet fisheries).

Differences in estimated stock composition were obtained for the purse-seine and gillnet test fisheries in July samples, where higher proportions of Great Central Lake sockeye salmon were observed in the gillnet fishery samples. Although the fishery samples came from different areas (the purse-seine samples were collected farther inland in Alberni Inlet than were the gillnet samples), the most likely explanation of the difference in estimated proportions of stock composition between the two gears is a difference in size selectivity. Sockeye salmon caught in purse seines in Barkley Sound are generally more variable in size and of smaller mean size than those caught in gill nets (Steer et al., 1986). Probably gill nets were more selective for Great Central Lake sockeye salmon than for Sproat Lake salmon. Thus, it is important to estimate stock contributions to a fishery catch based on samples collected with the type of gear employed in the fishery. Furthermore, the analysis of catch samples to estimate the stock composition of fish present in an area (as opposed to those caught in an area) will be biased to the degree that the sampling gear nonrandomly catches the fish that are present. The results of this study and other analyses (Beacham et al., unpubl. data) indicate that for salmonids, different gear types sample the various stocks in a stock mixture with very different efficiencies.

Differentiation among local spawning populations that are relatively stable over time provides the basis for applying biological markers to problems of salmonid fisheries management. This study confirmed our expectation that the level of differentiation observed at microsatellite loci among sockeye salmon of the three major lake systems draining into Barkley Sound is sufficient and stable to assess stock composition of the fishery catches. The abundance of highly polymorphic microsatellite loci in salmonid fish, the relative ease of nonlethal sample collection, and the moderate cost per fish for laboratory analysis combine to provide a technology that will become increasingly used in the assessment and management of salmonid fisheries.

Acknowledgments

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Direct validation of annual increments in the otoliths of juvenile southern bluefin tuna, *Thunnus maccoyii*, by means of a large-scale mark-recapture experiment with strontium chloride

Naomi P. Clear
John S. Gunn
Anthony J. Rees
CSIRO Marine Research
GPO Box 1538 Hobart, Tasmania, 7001, Australia
E-mail address (for N. P. Clear): Naomi.Clear@marine.csiro.au

Abstract.—From 1990 to 1996, during a large-scale tag-and-release program in the Great Australian Bight, 20,204 southern bluefin tuna (SBT), *Thunnus maccoyii*, were injected with strontium chloride ($SrCl_2$). The objectives of the marking experiment were to examine the efficacy of $SrCl_2$ as an otolith marker and to determine the periodicity of increment formation in SBT otoliths. Nine-hundred and sixty-one Sr-injected fish were recaptured and 616 otoliths were sampled from these; the high level of sampling success was attributable to a major liaison effort throughout the multinational SBT fishery. The same tag return rates for fish that were tagged and injected and for fish that were tagged only, indicated that the injection of strontium did not affect the survival rate of tagged fish. Strontium marks were detected with a Robinson detector or an energy dispersive spectrometer (EDS) (or with both) linked to a scanning electron microscope; 59 of the 67 otoliths from injected fish examined had discernible marks. The intensity of the strontium mark and the dosage rates were linked; a dosage of 100 mg Sr/kg fish weight is recommended to ensure easy identi fication of the strontium mark. Using the strontium marks, we established that in SBT with 1 to 6 increments in their otoliths, one increment is laid down per year at liberty. In the 59 marked fish that were examined, there was 100% agreement between the expected and observed number of increments after marking. These results, and the data from two supplementary tag returns from unmarked fish recaptured after long times at liberty, provide unambiguous evidence that increments on the otoliths of SBT are formed annually, to at least the age of 13 years. In addition, a recent study that used bomb-radiocarbon levels to estimate ages of older SBT has provided strong evidence that annual increments are deposited in the sagittal otoliths of SBT throughout life.

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Southern bluefin tuna (SBT), *Thunnus maccoyii* Castelnau, 1872, is a large, long-lived, migratory, pelagic fish with a circumglobal distribution between 30°S and 50°S (Caton, 1991). Its only known spawning ground is in the Indian Ocean south of Java, between 7°S and 20°S (Caton, 1991). Since it was first exploited in the 1950s the stock has declined dramatically to between 16% and 25% of its initial level (Sainsbury[1]). The fishery is currently managed by individual transferable quotas (ITQ) and the total allowable catch (TAC) is assessed each year.

Virtual population analysis (VPA) has been the main method of assessing the condition of SBT stock since 1980 (Murphy and Majkowski, 1981). The age structue of the population, a major input to VPA, has been estimated by converting lengths and weights to ages, using growth curves derived from tagging data (Hampton, 1991; Polacheck et al.[2]). To reduce the unmeasurable uncertainties that the estimates introduce into VPA assessments, a validated direct method for determining age was required.

In 1992 we began a study to develop reliable techniques for determining ages of SBT. Validation of assigned ages is critical in age estimation studies (Beamish and McFarlane, 1983; Smith, 1992; Secor et al., 1995); therefore a large-scale mark-recapture experiment was initiated to provide the basis for validating the age estimates. From the validation study we aimed to confirm the periodicity of the zones that were counted on the hard parts collected from SBT. The overall objective of these two studies was to develop a validated length-at-age key for the entire size range of the SBT population. We present details of the mark-recapture experiment and our evidence that increments in otoliths are formed annually.

[1] Sainsbury, K. 1993. What is happening to the southern bluefin stock? *In* W. Whitelaw and V. Mawson (eds.), Proceedings of the inaugural southern bluefish tuna science-industry-management workshop, Port Lincoln, Australia, p. 5–19. Commonwealth Scientific and Industrial Research Organization (CSIRO) Marine Research, GPO Box 1538 Hobart, 7001 Australia.

[2] Polacheck, T., K. Sainsbury, and N. Klaer. 1995. Assessment of the status of the southern bluefin tuna stock using virtual population analysis—1995. Paper SBFWS/95/17. First scientific meeting of the Commission for the Conservation of Southern Bluefin Tuna (CCSBT), Shimizu, Japan, 70 p. Commonwealth Scientific and Industrial Research Organization (CSIRO) Marine Research, GPO Box 1538, Hobart, Tasmania, 7001, Australia.

Previous attempts to estimate SBT ages directly, either did not attempt validation, or attempted it for only a few age classes. Hynd (1965) used scales to estimate ages of fish up to 80 cm fork length (FL) but did not attempt to validate his age estimates. Yukinawa (1970) counted up to eight rings on scales, using marginal increment analysis, to show that the rings form at the same time each year. Thorogood (1987) used otoliths to estimate age in fish from 42 to 167 cm FL and, using marginal increment analysis, was able to show seasonal band formation in what he called ages 2 to 4. Jenkins and Davis (1990) examined microincrements in the otoliths of SBT larvae between 3.5 and 12 mm standard length (SL) collected from the same cohort over consecutive days. From these microincrements, they validated daily increment formation and assigned approximate ages of 7 to 18 days to their samples.

In many age determination studies of other species of tuna, tetracycline has been used in marking experiments to validate daily increment formation in wild and captive tunas: e.g. yellowfin tuna, *Thunnus albacares* in the wild (Wild and Foreman, 1980; Wild et al., 1995) and in captivity (Yamanaka[3]); skipjack tuna, *Katsuwonus pelamis,* in the wild (Wild et al., 1995); black skipjack tuna, *Euthynnus lineatus,* in captivity (Wexler, 1993); and Atlantic bluefin tuna, *Thunnus thynnus,* in the wild (Inter-Am. Trop. Tuna Comm.[4]).

However, similar experiments using oxytetracycline (OTC) as a marker in SBT in the 1980s were less successful. In high proportion of OTC-injected fish, a mark failed to show up in the otoliths (Gunn[5]). Given this previous failure, and public health concerns over the use of tetracycline (in the USA, the Federal Drug Administration [US FDA] prohibits its use in wild fisheries), we selected strontium chloride ($SrCl_2$) as an alternative marker.

Strontium chloride is a nontoxic salt that occurs naturally in sea water. It is a component of some foods and is considered to be benign at the concentrations used as a marking agent (Sax and Lewis, 1987). Strontium is readily incorporated into the bloodstream of fish and, although not used previously on scombrids, it has been used successfully with other fish species to mark vertebrae (by introduction into food or in the surrounding water [Behrens et al., 1990]), and otoliths (by immersion and injection [Brothers, 1990]). Strontium is chemically and biologically similar to calcium. Because calcium and strontium ions have the same valency (2+) and a similar ionic radius (Ca, 0.099 nm; Sr, 0.113 nm), strontium readily substitutes for calcium during deposition of calcium carbonate.

The first two objectives of this study were 1) to evaluate whether intramuscular injection of strontium chloride resulted in effective and reliable marking of otoliths, and 2) to determine if strontium chloride injections increased mortality and, hence, affected recapture rates.

If successful and benign marking was demonstrated, we planned to use the strontium chloride marks to verify the accuracy of direct aging techniques by determining the periodicity of increment formation for as many year classes of SBT as possible.

Materials and methods

Tagging and marking

From 1990 to 1996, a total of 64,497 juvenile SBT in the Great Australian Bight were tagged and released. Of these, 20,204 tagged SBT were injected with $SrCl_2$ (Table 1). All fish were double-tagged (in case of "tag shedding") (Williams, 1992): strontium-injected fish were tagged with orange tags, fish that were not injected were tagged with yellow tags. When both orange and yellow tags were being deployed, an equal number of fish from targeted schools were chosen at random for injecting or not injecting. The smallest fish caught during the tagging program was 40 cm fork length (FL); we did not tag and inject fish smaller than this size because they were considered prerecruits, i.e. were not caught in the fishery. The lengths of orange-tagged fish ranged from 41 to 120 cm in FL. The return rates of yellow-tagged and orange-tagged fish were compared by using a chi-squared test to determine if they were significantly different.

The strontium chloride solution for injection was prepared in the laboratory. A stock solution of 1 g $SrCl_2.6H_2O$/mL was made up by dissolving 1 kg of analytical grade $SrCl_2.6H_2O$ crystals in 1 liter of distilled water, resulting in a 0.21 g/mL solution of Sr^{2+}. The solution was buffered to pH 7.0 with KOH and stored between 0°C and 4°C.

For rapid injections into large numbers of fish, a 5-mL automatic vaccinator fitted with a 0.2-mm needle was used. Flexible tubing connected the vaccinator to a plastic storage container that was either worn as a

[3] Yamanaka, K. L. 1990. Age, growth and spawning of yellowfin tuna in the southern Philippines. FAO, Indo-Pacif. Tuna Dev. Man. Prog. Working paper 90/WP/21, 87 p.

[4] Inter–Am. Trop. Tuna Comm. 1982. Annual Rep. for 1981, 303 p.

[5] Gunn, J. S. 1992. Progress report on strontium chloride marking of SBT during 1990–92 CSIRO–JAMARC tagging programs. Paper MWS4/WP–3. Fourth workshop of the southern bluefin tuna recruitment monitoring and tagging programs, Hobart, Australia, 9 p. Commonwealth Scientific and Industrial Research Organization (CSIRO) Marine Research, GPO Box 1538 Hobart, 7001, Australia.

Table 1

Numbers of southern bluefin tuna released with yellow tags and numbers of SBT injected with $SrCl_2$ and released with orange tags, and a summary of yellow and orange tag returns from 1990 to 1996. The number of returned yellow and orange tags released in each year of the tagging program, and the number of returned tags as a percentage of the total number released in the year (shown in parentheses) are shown. (The years of release and recapture are from October of one year to September of the next.) NS = not significant.

	1990–91		1991–92		1992–93		1993–94		1994–95		1995–96		1990–96 All release years	
	yellow tags *n*=6909	orange tags *n*=835	yellow tags *n*=4543	orange tags *n*=3595	yellow tags *n*=5907	orange tags *n*=5304	yellow tags *n*=8253	orange tags *n*=8251	yellow tags *n*=15,683	orange tags *n*=2219	yellow tags *n*=2998	orange tags *n*=0	yellow tags *n*=44,293	orange tags *n*=20,204
Fish recaptured														
1990–91	183 (2.65)	25 (2.99)	—	—	—	—	—	—	—	—	—	—	183	25
1991–92	180 (2.61)	19 (2.28)	84 (1.8)	55 (1.53)	—	—	—	—	—	—	—	—	264	74
1992–93	111 (1.61)	11 (1.32)	116 (2.6)	86 (2.39)	56 (0.95)	63 (1.19)	—	—	—	—	—	—	283	160
1993–94	62 (0.90)	6 (0.72)	102 (2.2)	76 (2.11)	130 (2.20)	90 (1.70)	50 (0.61)	47 (0.57)	—	—	—	—	344	219
1994–95	29 (0.42)	6 (0.72)	43 (0.95)	36 (1.00)	177 (3.00)	116 (2.19)	171 (2.07)	168 (2.04)	67 (0.43)	20 (0.90)	—	—	487	346
1995–96	2 (0.03)	0	6 (0.13)	6 (0.17)	29 (0.49)	17 (0.32)	86 (1.04)	89 (1.08)	94 0.60)	25 (1.13)	21 (0.70)	—	217	137
1990–96 All recapture years	567 (8.22)	67 (8.03)	351 (7.68)	259 (7.20)	392 (6.64)	286 (5.40)	307 (3.72)	304 (3.69)	161 (1.03)	45 (2.03)			1,779 (4.01)	961 (4.76)
Difference between yellow and orange tag returns	5.02 (0.83)		3.17 (0.94)		1.44 (0.61)		3.22 (0.40)		1.68 (0.39)				2.10 (0.56)	
(χ^2(*P*))	NS		NS		NS		NS		NS				NS	

back-pack or attached to the tagging cradle (Williams, 1992).

We injected the fish about 2 cm below the dorsal midline, in line with the center of the first dorsal fin. We occasionally observed a loss of the solution from the muscle, especially in larger fish. When this happened, we noted the loss and injected the fish a second time. To minimize loss of solution we carefully expelled all the air from the vaccinator so that air bubbles were not injected into the muscle.

To determine a suitable dosage rate for SBT, initial trials with strontium were made on three nontuna species in captivity: silver trevally, *Pseudocaranx dentex*, sand flathead, *Platycephalus bassensis*, and purple wrasse, *Pseudolabrus fucicola*, the largest fish weighing 500 g. The dosages were based on Brothers' (1990) immersion and injection trials with strontium on trout—100 mg/kg—and Wild and Foreman's (1980) tetracycline experiments on tuna—0.3 mL/kg or 27.5 mg/kg of fish. Dosages between 50 and 200 mg Sr/kg resulted in clear Sr marks on otolith sections and no mortalities (CSIRO, unpubl. data[6]). In the field, the length of the fish was measured to the nearest centimeter, but weight could only be estimated. We therefore chose the required $SrCl_2$ dose according to length (Table 2). These dosages were increased for fish longer than 90 cm when the marks on the first otoliths recovered from SBT of this size showed up faintly, indicating low strontium absorption. The dosages were adjusted so that at least 80 mg of Sr was injected per kilogram of fish.

Tag collection and otolith sampling

A critical factor in the experiment was the sustained effort, over many years, to recover otoliths from recaptured fish. An extensive campaign was conducted to explain the objectives of the marking experiment to the SBT fishing industry and to develop a protocol for

[6] CSIRO (Commonwealth Scientific and Industrial Research Organization) unpublished data. 1989. CSIRO Marine Research, GPO Box 1538, Hobart, Tasmania, 7001, Australia.

Table 2

Dosages for $SrCl_2$-injected fish, based on length and weight of fish. FL = fork length.

FL (cm)	Approx. wt (kg)	Dose 1990–92			Dose 1993–96		
		$SrCl_2$ (mL)	Sr (mg)	mg Sr/kg fish	$SrCl_2$ (mL)	Sr (mg)	mg Sr/kg fish
40–50	1.5–3.0	2	400	130–270	2	400	130–270
51–70	3.0–7.0	3	600	86–200	3	600	86–200
71–80	7.0–10	4	800	80-114	4	800	80–114
81–90	10–15	5	1000	67–100	5	1000	67–100
91–95	15–18	6	1200	67–80	7	1400	78–93
96–100	18–21	7	1400	67–78	9	1800	86–100
>100	>21	8	1600	76	12	2400	114

collecting the samples. Posters and information notes, in Japanese and English, were distributed throughout the fishery. Rewards were offered for the return of tags and a substantial bonus for allowing scientists to sample otoliths from orange-tagged fish. To aid in otolith recovery, kits containing large orange disks were distributed to the crew of Japanese vessels; when an orange-tagged fish was caught, the disks were attached to the fish to clearly identify it in the freezers.

Given the high value of SBT on the Japanese sashimi market, it was essential that otoliths could be sampled without affecting the external appearance of the fish. Using a modification of the technique described by Thorogood (1986), we removed from the skull 35–44 mm cores that contained the semicircular canals and sagittal otoliths with a holesaw attached to a drill. The points of entry for the cores were over the basioccipital plates, which are anterior to the first vertebra and immediately lateral to the junction of the skull and first vertebra; this area was exposed when the tuna were cleaned and dressed—a process which removes the gill-filaments and surrounding tissue and most of the opercular flaps. By drilling through each of the plates in the direction of the back of the opposite eye, the cores coalesced at a point beyond the sagittal otoliths and could be removed easily from the skull, leaving no external mark on the fish (Fig. 1) Sagittal otoliths were removed from the cores, cleaned in distilled water, and dried at 28°C.

Age estimates

An age estimate was made from the otoliths before we attempted to identify a Sr mark. Increments on the whole sagittal otoliths comprised two zones: an opaque (assumed to be fast growth) and a narrower translucent zone (assumed to be slow growth) that appeared dark under a dissecting microscope with reflected lighting and a black background. Following Thorogood's (1987) method, one of each pair of sagittae was burned on a 400°C hot plate until it turned golden brown. The color change was greater in the translucent zones, making them more visible (Fig. 2A). A digital image of the burnt otolith was taken (using the public domain "NIH Image" program[7] and a video camera mounted on a Wild M5A dissecting microscope) and measurements were made of the otolith length and of the distance between the primordium and the inside of the translucent zones along both the postrostral (PR) and transverse axes. We use terminology that is currently widely accepted (Secor et al. [1992]; Kalish et al. [1995]; Stequert et al. [1996]). For each specimen, the reader made three independent age estimates by counting the number of increments obvious on the distal surface of the sagitta. These were made without reference to either the length of the fish or the time that the fish was at liberty after tagging and injection.

Detection of strontium marks

Scanning electron microscope (SEM) with a Robinson backscatter detector In the early stages of the project we used a Robinson backscatter detector coupled to a Philips 515 SEM for detecting the strontium-rich band in the otoliths (which we refer to as "the strontium mark"). The Robinson detector visualizes differences in the total atomic weight (Z) across a specimen. Because a strontium mark within the calcium carbonate contains a higher concentration of Sr, and hence a higher Z than surrounding uncontaminated calcium carbonate, it appears as a weak to intense bright band across the growth axis of the section (Figs. 2B and 3). The

[7] Rasband, W. 1994. NIH Image, vers. 1.54. U.S. National Institutes of Health. [Available from the Internet by anonymous ftp from zippy.nimh.nih.gov or as a floppy disk from NTIS, 5285 Port Royal Rd., Springfield, VA 22161, part number PB93-504868.]

intensity of the band depends on the magnitude of the difference in Z between the two portions of the otolith.

This kind of analysis requires a flat, polished surface; therefore we sectioned the sagittal otoliths either along the postrostral axis to produce an oblique longitudinal section (LS), or along a transverse axis. The rostral axis often shows clear increments, but we did not find distinct Sr marks in this part of the otolith. The sections were ground and polished following Gunn et al.'s (1992) methods and an evaporated carbon coat (25–30 nm thick) was applied to the sections to minimize charging in the SEM. The position of the Sr mark along the axis was measured with the vernier attached to the SEM stage drives (Fig. 2B).

Energy-dispersive spectroscopy (EDS) In the later stages, an EDS x-ray microanalysis system became available and we used it to confirm the presence of Sr in the bright bands, and also to detect Sr marks in unsectioned otoliths (the use of unsectioned otoliths decreased the preparation time required for SEM analysis). The system consisted of a Link 133 eV Si(Li) detector with light element capability and a Thomson Scientific "WinEDS" PC-based analyzer attached to a Philips 515 SEM. Before x-ray analysis, whole (unsectioned) otoliths were acid-etched along the postrostral axis from the surface with 1 N and 3.5 N HCl, to expose the growth plane, then rinsed in bleach and distilled water, and dried. To minimize charging in the SEM, the otoliths were dipped in a dilute carbon DAG solution (approximately 1:50 with dichloroethane) immediately before analysis. Strontium marks were detected by operating the SEM in "spot" mode and searching for a point or zone where a significant Sr signal was detected on the x-ray microanalyzer (typically, a strong peak at 1.81 keV in the spectrum, corresponding to emission of Sr Lα x-rays). When a strontium mark was detected, its position along the PR axis was measured and the mark photographed either with conventional SEM photography (Fig. 4) or with a rapid, low-grade video print, which also showed the features of interest. To confirm that the suspected mark was strontium-rich, two plots of the x-ray spectra from the otolith were taken: one on the strontium mark and one just before the mark. Acceptable evidence of the correct identification of a strontium mark was considered to be the presence of an enhanced Sr level in the area analyzed, together with an absence of Sr (except for background levels) immediately before the area (Fig. 5).

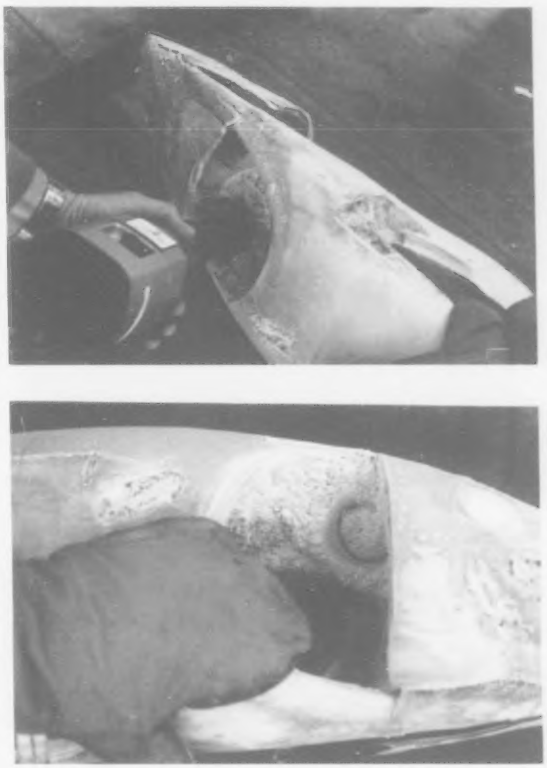

Figure 1

The drilling technique used to extract otoliths from southern bluefin tuna destined to be sold as "whole" fish.

The measurements of increments on the whole otoliths, and the strontium mark in sections or whole otoliths, were made along the same axes without reference to the other. This procedure enabled us to compare the number of increments observed after the strontium mark with the number expected, calculated from the known time at liberty after tagging.

Quantitative EDS analyses for linescans were carried out on carbon-coated polished sections in the Philips 515 SEM operated at an accelerating voltage of 20 kV, by using a focused electron beam of 0.15 µm diameter and analysis times of 60–200 seconds. The effective area analyzed by the beam was larger than the diameter of the beam itself because the beam spreads within the specimen after entry; examination of superficial beam damage to specimens after analysis suggested that the area analyzed by the beam is in the order of 2 µm diameter. Elemental concentrations were calculated by reference to appropriate standards

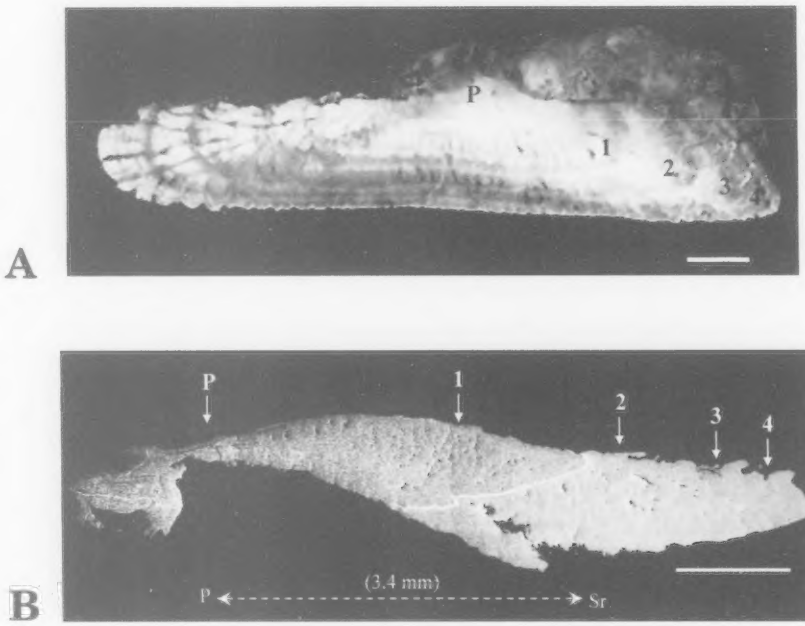

Figure 2

Sagitta (specimen OB 764) from a fish tagged and injected in March 1994 at 57 cm FL and recaptured in February 1997 at 111 cm FL. (**A**) Four increments were counted on the whole, burnt otolith and measured from the primordium (P) to the beginning of each translucent zone apparent on the distal surface along the postrostral axis at 2.2 mm, 3.6 mm, 4.4 mm, and 4.9 mm. (**B**) The sagitta was sectioned through the postrostral axis and viewed in the SEM: the micrograph shows the strontium mark which was located 3.4 mm from the primordium and the positions of the translucent zones measured on the otolith before it was sectioned. Scale bars: 1 mm

(calcite and celestite for Ca and Sr, respectively), with the "WinEDS" software.

Results

Of the 20,204 fish injected with $SrCl_2$ between 1990 and 1996, 9614 had been recaptured and 616 sets of sagittal otoliths were recovered from these by 1 January 1996. Seventy sets of otoliths were chosen for the validation study, selected from the range of size classes in the recaptures—fish of 45 to 102 cm FL at release and 57 to 133 cm FL at recapture—and from the range of times at liberty. Age estimates were made from 67 of the 70 otoliths; three sets of otoliths were excluded from the experiment because the increments on the whole otoliths were either ambiguous or uninterpretable and the reader could not give an age estimate with confidence.

Recapture rates of orange-tagged fish and recovery of otoliths

There were no statistically significant differences between the return rates of yellow tags (from fish not injected with $SrCl_2$) and orange tags (from fish injected with $SrCl_2$) released in all years of the program (χ^2=2.10, P=0.56) nor between the return rates of yellow tags and orange tags for any of the release years (Table 1). The number of otoliths recovered, as a percentage of orange tags recaptured, varied between 20%, in the first year of the tagging program, and 88% in the final year (Table 3); overall, otoliths were recovered from 65% of the orange-tagged fish that were recaptured.

Detection of Sr marks in the otoliths of orange-tagged fish

The otoliths removed from fish injected with strontium chloride typically showed a bright band in back-

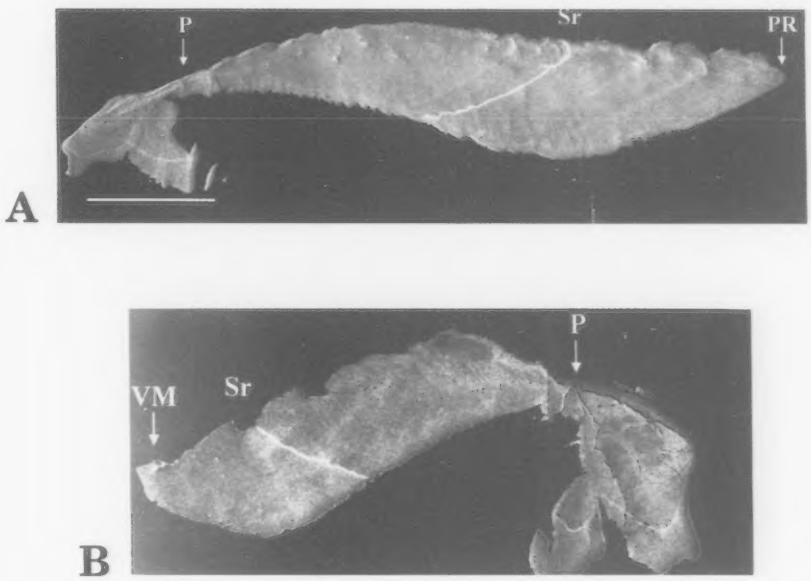

Figure 3

SEM micrographs showing strontium marks apparent as bright bands on sections taken from sister otoliths of specimen OB 102. (**A**) An oblique longitudinal section through the postrostral axis on which the primordium (P) and postrostrum (PR) are marked. (**B**) A transverse section on which the primordium and ventral margin (VM) are marked. Scale bars: 1 mm

Table 3

The number of tagged fish recaptured, and the number of otoliths recovered from orange-tagged fish during each year of the tagging program. (Data to 16 February 1996. The years of release and recapture are from October of one year to September of the next.)

Recapture year	Yellow-tagged fish recaptured	Orange-tagged fish recaptured	Otoliths recovered (otoliths recovered as a percentage of the orange-tagged fish recaptured)
1990–91	183	25	5 (20)
1991–92	264	74	49 (66)
1992–93	283	160	94 (59)
1993–94	344	219	107 (49)
1994–95	487	346	248 (72)
1995–96	238	137	120 (88)
Total	1799	961	623 (65)

scattered electron images of polished sections through appropriate growth planes (e.g. the oblique longitudinal section along the PR axis). The visibility of the Sr mark (brightness in the backscattered electron image) was highest in fish that had been relatively small at the time of injection (e.g. 50–55 cm FL). An example is specimen OB 102 (Fig. 3), which measured 49 cm at time of release and was injected with 2 mL $SrCl_2$ solution. This bright band was frequently associated with a local growth interruption immediately before the band, presumed to be a tagging check. This check was sometimes seen on the whole, etched otoliths (Fig. 4) but, although apparent in the SEM at high magnifications, it was not discernible from the other surface

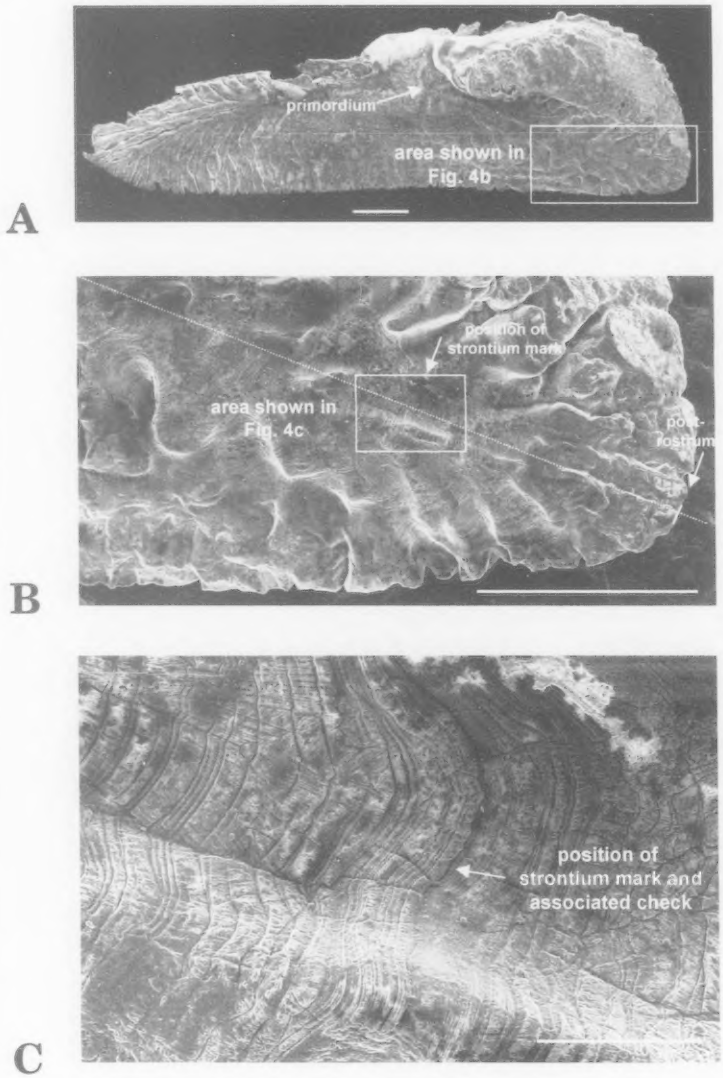

Figure 4

SEM micrographs of a carbon-coated whole etched otolith of specimen OB 127, showing the position of the strontium mark and the check associated with tagging and injection. The dotted line (B) indicates the axis along which the position of the strontium mark was measured in relation to the primordium and the postrostrum. Scale bars: 1 mm (**A** and **B**), 0.1 mm (**C**).

features on the whole otolith when increments were counted. We could not identify Sr marks on vertebral sections from fish injected with $SrCl_2$.

The presence of strontium in the bright bands was demonstrated by EDS spectra, which showed a strong peak of strontium Lα x-rays when the electron beam was directed to the Sr mark, in contrast with very low (background) levels in the regions of the otolith preceding the mark (Fig. 5). The relative levels of strontium and calcium in the bright band were further confirmed by inspection of the difference spectrum obtained by subtracting a spectrum acquired in the area

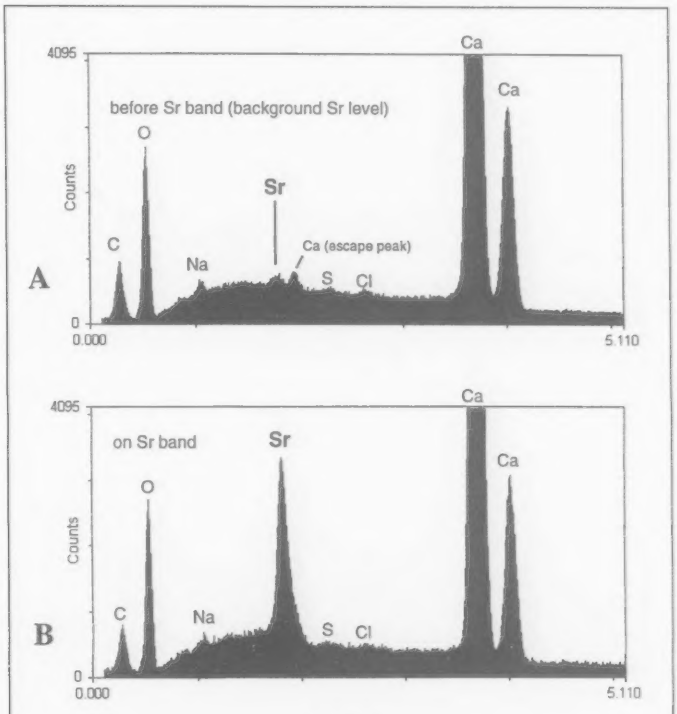

Figure 5

Examples of EDS spectra from a sectioned SBT otolith showing peaks due to background levels of Sr (**A**) and enhanced Sr levels associated with the strontium mark (**B**). Spectra similar to that shown in Figure 6B were used to positively identify the location of strontium marks in sections and etched whole otoliths (see text for further details).

preceding the mark (e.g. Fig. 5A) from one acquired on the bright band (Fig 5B), which demonstrated that strontium levels were enhanced and calcium was reduced in the bright band; however, no increase in chlorine was apparent. A quantitative EDS linescan across a bright band in one specimen, SBT OB 96, sectioned in oblique LS along the PR axis and analyzed along the direction of maximum observed growth, revealed "background" levels of 0.1% to 0.25% Sr by weight up to 5 microns before the band and a measured peak of 7.1% Sr on the band, falling to 3.5% (50% of peak level) 6 µm after the start of the band, and 0.7% (10% of peak level) approximately 15 µm after the start of the band. There is some indication of continuing slightly elevated Sr levels out to around 50 µm beyond the band, although visibility of these levels is at the limits of the EDS technique (Fig. 6).

Accompanying the measured maximum 7.1% increase in Sr level in the bright band is a fall in measured Ca concentration from 39% to 40% before the band to a minimum of 35.5% on the band—a decrease of 3.5–4.5% in absolute value or 10% relative value. Within the limits of accuracy of the EDS technique, this decrease in calcium concentration supports the theory that Ca atoms are being replaced by Sr atoms in the atomic structure on a 1:1 basis, each Sr atom being approximately twice as heavy as a Ca atom. Calculation of the increase in mean atomic number of the specimen resulting from a 7% increase in Sr and a 3.5% decrease in Ca gives a value of approximately 104 for the Sr-enriched zone. This value compares with 100 for the unaltered $CaCO_3$—a difference resolvable with backscattered electron imaging on the SEM on a suitably polished and coated specimen. The extent of the visible bright band in this specimen (OB 96) coincided with measured Sr levels in the range of 5–7%; thus it is possible that elevated Sr concentrations in the range of 0.5–5% may not be detectable by backscattered imaging although they should still be detectable by EDS. The EDS system is also essential for testing the identity of weak bright bands in sectioned specimens when it is not clear from

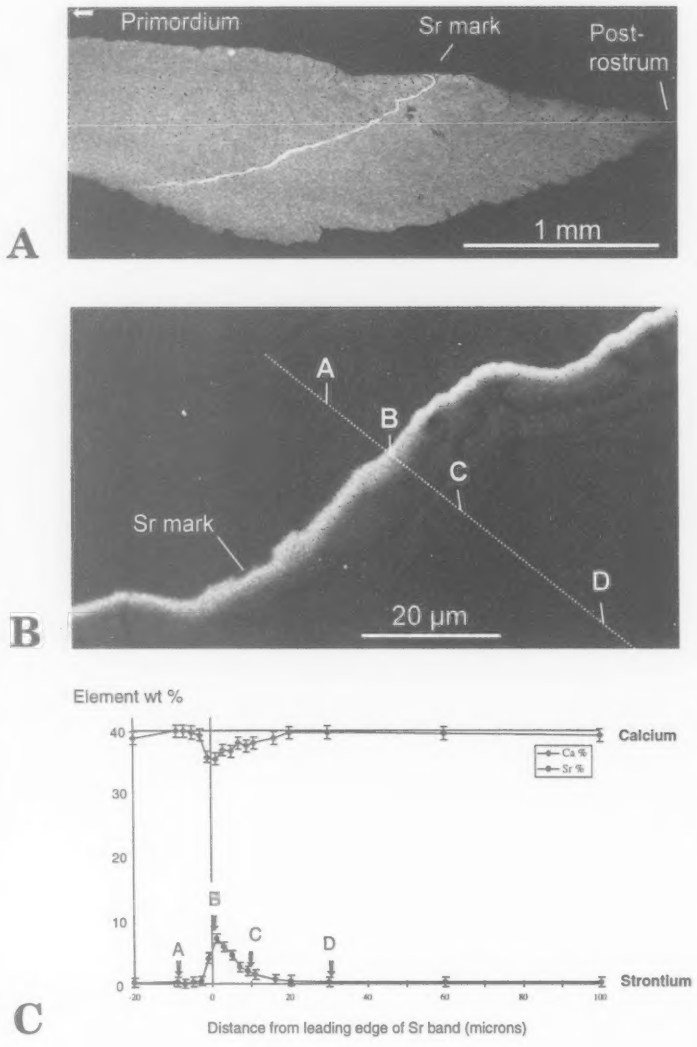

Figure 6

Details of the strontium mark on polished LS of specimen OB 96. Backscattered SEM images showing overall location of mark (**A**) and detail of region analyzed for Sr and Ca levels (**B**). Dashed line indicates transect followed for x-ray microanalysis (**C**): Measured variation in Sr and Ca levels along transect shown in B; points A–D indicate representative positions along the transect to enable comparison of sections B and C.

the backscattered imaging which band, if any, is a strontium mark.

Reliability of marking through injection of strontium

Of the 67 otoliths from which an age was estimated, strontium marks were detected with the Robinson detector in 19 of the 20 sectioned otoliths (95% detection rate) and with EDS in 40 out of 47 whole otoliths (85% detection rate). Strontium marks were as visible in the oblique longitudinal section (postrostral axis) as in the transverse section (Fig. 3). However, as increments are more widely spaced along the postrostral axis, we generally used the postrostral axis on the whole otolith for measuring the

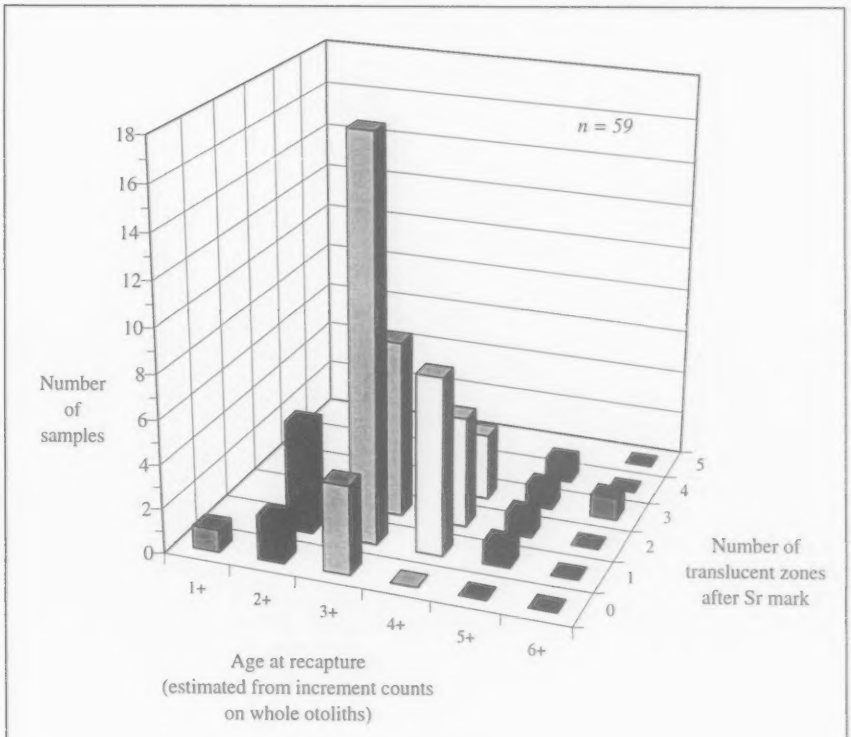

Figure 7

The number of strontium-marked otoliths used to validate age estimates that were made by counting increments on whole otoliths. The number of translucent zones observed after the strontium mark is shown for each age class. For all otoliths analyzed, the number of translucent zones counted after the Sr mark equalled the number that were expected from the period at liberty after fish were tagged and injected with $SrCl_2$.

position of translucent zones and for locating the strontium mark, either on a section (on the Robinson detector), or from the whole, etched otolith (with EDS).

Early in the experiment we found that the Sr marks in fish tagged and injected when they were 90 cm FL or larger were consistently fainter than in smaller fish. The concentration of Sr in the Sr marks of large fish was also significantly lower than in smaller fish caused, possibly, by loss of some of the solution during injection. To overcome this, dosages for large fish were increased in 1993 (Table 2), after which the bands were markedly more intense and easier to detect.

There was no apparent correlation between the time at liberty (i.e. time between Sr injection and recapture) and the intensity of the Sr marks; the longest time at liberty for a fish from which otoliths were analyzed for Sr was 1638 days. There was also no correlation between the intensity of the Sr marks and the delay between otolith recovery and analysis. Unlike tetracycline, which is photosensitive, the strontium mark did not fade after exposure to light.

Validation of annual increment formation from Sr marks

The 59 fish in which Sr marks were located ranged from 45 to 102 cm FL at the time of release, which corresponds to estimated ages of 1 to 4 (Gunn et al.[8]). Times at liberty ranged from 8 to 1638 days. The oldest recaptured fish was estimated to be 6+ years old; it had been at liberty for 1242 days (over 3 years).

[8] Gunn, J., N. Clear, T. Carter, A. Rees, C. Stanley, J. Kalish, and J. Johnstone. 1995. Age and growth of southern bluefin tuna. Paper SBFWS/95/8. First scientific meeting of the Commission for the Conservation of Southern Bluefin Tuna (CCSBT), Shimizu, Japan, 37 p. Commonwealth Scientific and Industrial Research Organization (CSIRO) Marine Research, GPO Box 1538 Hobart, 7001 Australia.

We counted six translucent zones on the otolith and the Sr mark occurred between the third and fourth zones (Fig. 7).

For all otoliths examined, there was agreement between the number of increments observed after the strontium mark and the number of increments expected, calculated from time at liberty. Thus, the annual periodicity in formation of increments 2 to 6 was validated for the otoliths analyzed. Because we were unable to tag young-of-the-year fish, in which the first translucent zone on the sagitta had yet to form, we could not determine when this translucent zone is laid down and when the formation of the first increment is completed. However, studies of daily microincrements (Itoh and Tsuji, 1996; Rees et al.[9]) have calculated that the approximate size at age 1 is 50 cm. We found otoliths of 50-cm fish had one increment.

Of the otolith increments counted, the first translucent zone was typically the most difficult to measure. The beginning of the first translucent zone occurred between 2.2 and 3.2 mm from the primordium along the postrostral axis, the most commonly used axis for analysis. Rees et al.[9] found microincrements in this region to be narrower than those deposited earlier, indicating a period of slow growth of the fish.

Additional validation of annual increment formation from tagged fish at liberty for extended periods

During the course of our experiment, two fish tagged by CSIRO in the 1980s were recaptured and their otoliths sampled. From lengths at first release of 45 cm and 82 cm FL, the fish had grown to 163 cm and 162 cm after being at liberty for 9 years, 7 months, and 10 years, 8 months, respectively. From the age-length key developed by Gunn et al.[8] we calculated that the 45-cm fish tagged in 1983 was one year old when tagged, whereas the 82-cm fish tagged in 1984 was two years old. The ages at recapture of these two fish were estimated from transverse sections through the primordium of the sagittal otoliths. Eleven increments (opaque and translucent zones) were counted on the otoliths from the fish released as a one-year-old and caught 9.58 years later; 13 increments were counted in the fish released as a two-year-old and recaptured 10.75 years later.

[9] Rees, A. J., J. S. Gunn, and N. P. Clear. 1996. Age determination of juvenile southern bluefin tuna, *Thunnus maccoyii*, based on scanning electron microscopy of otolith microincrements. *In* J. Gunn, N. Clear, T. Carter, J. Farley, A. Rees, and C. Stanley, Appendix 1: The direct estimation of age in southern bluefin tuna. Second scientific meeting of the Commissionfor Conservation of Southern Bluefin Tuna (CCSBT), Hobart, Australia. 26 August–5 September 1996, 22 p. Commonwealth Scientific and Industrial Research Organisation (CSIRO) Marine Research, GPO Box 1538, Hobart, Tasmania, 7001 Australia.

Discussion

Validation

This study demonstrated that, in the sagittae of SBT, the second through sixth increments, are deposited annually. This validation is independent of when the marked fish were tagged or recaptured. Because daily age estimates have been used to demonstrate that the first major increment in the sagitta forms in the first year of life (Rees et al.[9]), the annual formation of translucent zones appears to hold for the first six increments in SBT sagittae—corresponding to fish up to approximately 133 cm fork length.

The close agreement between increment counts on otoliths and the sum of age-at-tagging and time-at-liberty for two fish tagged in the 1980s and recaptured in the 1990s indicated that increment formation continues to be annual in fish up to at least 13 years old. Further evidence that increments in SBT sagittae are formed annually throughout life has been provided by a recent comparison of increment counts with age estimates derived from levels of bomb-radiocarbon in the early growth zones of sagittae (Kalish et al. 1996). This study reports close agreement between the two methods of estimating age for fish up to 34 years old.

Three sources of data—those from our marking experiment, the increment counts for two fish at liberty for over a decade, and the bomb radiocarbon data—provide strong evidence that seasonal changes in growth are expressed as clearly identifiable annual increments in the sagittal otoliths of SBT. These increments can be used to estimate the age of individual fish at any point in their lifespan.

Prior to our studies, Yukinawa (1970, using scales) and Thorogood (1987, using otoliths) used marginal increment analyses to demonstrate the annual check or translucent band deposition in fish they considered to be between 2 and 4 years old. Their results differ from ours only in the identity of year classes; their two- to four-year-olds correspond to our one- to three-year-olds. The difference in scale readings derives from Hynd's (1965) observation of two "checks" on the scales of new recruits (approximately 50 cm FL) to the Western Australian fishery. Interpretation of otolith microincrements (Itoh and Tsuji, 1996; Rees et al.[9]) indicates that these fish are only one year old. Unequivocal validation of these estimates is not possible at this stage because samples from prerecruits were not available to either Hynd or Yukinawa and we were not able to tag and mark prerecruit fish. In a number of other *Thunnus* species however, 50–60 cm fish were found to be around one year old (Uchiyama and Struhsaker, 1981; Wild, 1986; Foreman, 1996) and our counts of otolith microincrements and the data based on their inter-

pretation are consistent with this age. Therefore, we believe that the interpretation of Itoh and Tsuji (1996) and Rees et al.[9], that 50-cm-FL fish are one-year-olds, is most likely correct.

The identification of fish that we considered to be one-year-olds as two-year-olds was made by Thorogood in his 1987 study. However, we have found no evidence of two increments in the otoliths of new recruits. The early zones on all axes of otolith growth are difficult to read on some otoliths, and the increments in these areas are less distinct than those deposited later. In some fish a poorly defined "band" is also present very close to the primordium (within 2 mm along the postrostral axis). Although these two factors may confuse an inexperienced reader, Thorogood makes no mention of difficulty in reading the first increment. An alternative explanation for Thorogood's interpretations may be that his readings were influenced by the findings of Hynd (1965) and Yukinawa (1970) that were based on scales, because their estimates of age at recruitment were entrenched within the dogma of SBT population dynamics current in the 1970s and 1980s.

It has been hypothesized that more than one translucent zone forms per year in the otoliths of mature Atlantic bluefin tuna, *Thunnus thynnus* (Berry et al., 1977; Lee et al., 1983). In females, one translucent zone may correspond to a winter slow-growth period, the other to a spawning period (Lee et al., 1983). In the two large, tagged fish examined in our study, only one opaque and one translucent zone were deposited per year throughout life. The outer increments (i.e. those assumed to be deposited after sexual maturation) were consistent in both their width and optical density and were visually equivalent to the increments described by Lee et al. (1983), comprising a wide opaque region and a narrow translucent area that, on a black background, appears dark under reflected light. Occasionally, there appeared to be two translucent zones closer together than normal and, if these bands coalesced at the margin, they were counted as part of the same increment. These may be equivalent to the bands described by Berry et al. (1977) who hypothesized that a pair of these paired bands represented an annual increment. The close agreement between otolith increment counts and bomb-radiocarbon age estimates for mature SBT up to 34 years old (Kalish et al., 1996) supports our hypothesis that one increment, comprising one translucent and one opaque zone, continues to form per year, as does the consistency of the width and optical density of increments deposited after sexual maturation in the otoliths aged by Kalish et al. (1996). In summary, there is no significant evidence to suggest that mature female SBT deposit two translucent zones per year. In this regard our findings are similar to those of Hurley and Iles (1983) and Hurlbut and Clay (1988) for *T. thynnus*; they found, albeit in the absence of direct validation, that a single translucent zone is laid down per year in medium- and giant-size classes.

The use of strontium chloride to mark otoliths of large fish

This study has shown that intramuscular injection of strontium chloride leaves a distinct mark on the otoliths of SBT that is clearly visible as an SEM backscatter image in the Robinson detector. In the 20 otolith sections from Sr-injected fish that we examined, 95% had detectable marks. On this basis, we conclude that the compound is an efficient marker. Success of OTC as a marker at this rate of detection (95%) leads to high mortalities (McFarlane and Beamish, 1987). The high detection rates and lack of evidence of mortality for $SrCl_2$ are not surprising. This mineral occurs naturally in sea water, the mean concentration being 3.8–8.2 ppm (Carriker et al., 1991) or 0.09 mM/kg (Bruland, 1983), and both Sr and Cl are major constituents of the otoliths of SBT (Gunn[5]). When $SrCl_2$ is injected into the muscle it is taken up into the blood stream and incorporated in the endolymph and then the otolith, substituting for Ca within the $CaCO_3$ portion of the aragonite. The combined weight fraction of Ca and Sr (approximately 42%) within the otolith does not change as a result of the injection. However, the Ca:Sr ratio changes from 250:1 before injection to as low as 5:1 during the period over which the Sr spike induced by the injection is metabolized. At a distance of 6 μm beyond the injection mark, the Sr levels have dropped to about 50% and at 15 µm to 10% of peak values (Fig. 6). These distances correspond to time periods in the order of 2 and 5 days, respectively, based on median growth rates of around 3.0 µm/day estimated along this axis (Rees et al.[9]).

Detecting Sr marks on whole otoliths by using EDS was possible because the growth plane of tuna otoliths lies near the surface of the distal face. In otoliths of young fish, etching will expose the growth plane, so that sectioning is not required. Although this method of detection was slightly less successful (85%), it had two advantages. First, the preparation time was around half that required to prepare sections suitable for the Robinson detector. Second, the age estimate and measurements of increments were made along the postrostral axis on whole otoliths from the smaller fish (up to six years old), and the method of locating the strontium mark by EDS meant that the position of the strontium mark was measured along the axis in the same plane. With the Robinson detector, the same axis was measured but in cross section.

In fish older than about 6 years, the increments deposited on the margin can be unclear on whole oto-

liths; therefore transverse sections are used to determine ages of older fish (Gunn et al.[8]). In the future, as strontium-marked otoliths are returned from older fish that have been at liberty for longer periods, we will locate the strontium mark in transverse sections with the SEM and increase the number of increments that have been validated.

The recapture rates of orange-tagged and injected SBT were not significantly different from the recapture rates of yellow-tagged SBT; therefore the Sr injections apparently did not affect survival rate. Although the dosages of Sr varied between 65 and 250 mg/kg of fish, there is no evidence to suggest that higher doses increased mortality because Sr-injected fish with the highest doses were among those recaptured. A direct relation between the dosage and the intensity of the mark had been found in trials with three other species in 1990–91 (CSIRO, unpublished data[6]). The increased dose for large SBT resulted in much more distinct marks on their otoliths, which have a larger surface area than that in smaller fish. The less distinct marks could also be attributable in some large fish to a loss of strontium solution from the muscle after injection. Although more solution was injected if a loss was noticed, there may have been further loss of solution after the fish was returned to the water, resulting in a less effective dose. Thus, as a general guideline, we recommend a dose of 100 mg Sr/kg for marking otoliths in SBT. We note, however, that tissue area around the injection should be observed to ensure that there is no loss of injected solution from the muscle tissue.

The problem of detecting indistinct marks that result from low dosage levels are eliminated by using $SrCl_2$ as a marker. Unlike fluorescent marking, where it is very difficult to evaluate faint or ambiguous marks objectively (particularly if they are close to the outside edge of the otolith), it is possible to evaluate Sr marks objectively by x-ray analysis. Because the concentrations of the Ca and Sr on the Sr mark are high, very simple energy dispersive spectroscopy systems, which are available in many SEM facilities, can be used. Although not a trivial procedure, x-ray analysis requires preparation methods similar to those used for examining fluorescent markers and can usually be contracted to facilities at a low cost. Given the often substantial investment in tagging programs, and the common combination of low recapture rates and even lower otolith sampling rates, every sample is extremely valuable in a marking experiment. The safety net of chemical analysis is thus very advantageous.

Comparison of strontium and fluorescent markers

At the beginning of this project we chose strontium chloride over the more commonly used fluorescent markers because previous work on SBT with oxytetracycline had been unsuccessful. Although immersion in high concentrations of strontium or feeding with strontium-laced food (or both) had been used successfully for marking hard parts of larvae and juveniles of hatchery-reared salmon (Behrens Yamada and Mulligan, 1982; 1990), salmonids and a variety of tropical fish species (Brothers, 1990) and squid (Hurley et al., 1985), strontium had not previously been used to mark otoliths of large fish. On the basis of his experiments, Brothers (1990) concluded that, for mass marking of fish in captivity, detection of strontium marks was expensive and involved more difficult preparation than did fluorescent markers and other marking techniques such as thermal inducement (Volk et al., 1990).

Brothers' (1990) comment on expense is certainly pertinent but the expense of analyzing marked otoliths is often a small fraction of the cost of a marking experiment, particularly one where large numbers of fish have been tagged, injected, and released. Perhaps most important in the cost equation should be the rate of success of detecting marks in the otoliths of marked fish rather than the comparative cost of analysis. In otoliths from large tuna whose time at liberty has been long, the strontium marks are covered by a large amount of otolith material deposited after the time of injection. Sectioning is necessary for either marker; thus preparation times in these cases are much the same. For smaller tuna and those at liberty for short periods, fluorescent markers can be detected in the whole otolith, whereas detection of strontium without an EDS system would require sectioning, which would increase preparation time. The equipment for fluorescent markers is cheaper and comprises a light microscope equipped with an ultraviolet illumination source and filters to match the wave length of the fluorescence emitted from the marker when excited by the light source (see Wild and Foreman, 1980). For strontium, an SEM equipped with a Robinson detector is the minimum requirement; an EDS system is a useful extra. Although an SEM is a common apparatus in large research laboratories, hourly charges to the user can be high, although we have found that, with well prepared specimens, as many as four otoliths can be examined and analyzed per hour with an SEM.

Apart from preparation time and costs, strontium marking for age validation has clear advantages over fluorescent marking. One benefit of a technique that requires both a light microscope and an SEM is that measurements of increments and strontium marks are independent: the strontium cannot be detected in whole otoliths under the light microscope and the annual increments cannot be observed in the SEM. Allergic reactions by humans to compounds such as oxytetracycline have led the U.S. FDA to ban their use

in commercial fisheries. Strontium chloride, on the other hand, is regarded as safe for human consumption because it is a salt with a low order of toxicity (Sax and Lewis, 1987). It is even used in toothpaste by some manufacturers (e.g. "Sensodyne"). Strontium chloride, unlike fluorescent markers such as oxytetracycline, is not photosensitive. Neither the marking solution nor the marked otoliths need to be stored in the dark, and the mark does not fade with exposure to light or with time. In our study, strontium marks were as evident in fish that had been at liberty for long periods as in fish recaptured soon after release.

In summary, strontium chloride injection has proved to be a very successful way to mark the otoliths of southern bluefin tuna: 95% of those marked and recaptured in this study had detectable Sr marks in sectioned otoliths. This high "success rate," the harmless nature of $SrCl_2$ to both fish and humans, the capacity of EDS to positively identify the strontium mark, the insensitivity of the strontium mark to light, and the longevity of the strontium mark indicate that it should be seriously considered by those interested in large-scale marking experiments on commercial fishes.

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Reproduction, embryonic development, and growth of the porbeagle shark, *Lamna nasus*, in the southwest Pacific Ocean

Malcolm P. Francis
National Institute of Water and Atmospheric Research
P.O. Box 14-901
Wellington, New Zealand
E-mail address: m.francis@niwa.cri.nz

John D. Stevens
CSIRO Marine Research
G. P.O. Box 1538
Hobart, Tasmania, Australia

Abstract.—Porbeagle sharks, *Lamna nasus*, are caught in large numbers as bycatch in tuna longline fisheries in the southwest Pacific Ocean. Information on reproduction, embryonic development, and size and sex composition was collected by scientific observers from New Zealand and Australian waters, and supplemented with data from other sources. Most sharks were juveniles less than 150 cm fork length (FL), and length-frequency distributions showed 3–5 modal peaks that we interpret as age classes. Juveniles grow linearly and rapidly (16–20 cm per year), reaching 110–125 cm FL in three years. Females mature at around 165–180 cm. Litter size is usually four embryos and parturition probably peaks in June–July (winter). This finding contrasts with data for North Atlantic porbeagles which give birth in spring–summer. Embryos grow about 7 cm per month, and are born at 58–67 cm FL. The gestation period appears to be about 8–9 months, but there is considerable variability in embryo length at any one time, suggesting an extended mating period. Embryos are nourished by oophagy, and develop a grossly distended abdomen as their "yolk stomach" fills with ova. Small embryos have fang-like functional teeth that tear open egg capsules to release the contained ova. The fangs are shed at 34–38 cm FL. The weight of yolk in the stomach peaks at 30–42 cm FL, and accounts for up to 81% of total body weight. Waste products of yolk digestion accumulate steadily in the spiral valve throughout gestation, and the liver reaches its maximum size in near-term embryos as excess energy from yolk digestion is stored for postnatal use.

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The porbeagle, *Lamna nasus* (Bonnaterre, 1788) is a pelagic mackerel shark (family Lamnidae) that inhabits cool, temperate oceans. It occurs in the North Atlantic Ocean and in a circumglobal band in the southern Pacific, Atlantic and Indian Oceans (Compagno, 1984; Last and Stevens, 1994; Yatsu, 1995). It is absent from the North Pacific, where it is replaced by its closest relative, the salmon shark (*Lamna ditropis*).

Lamnid sharks produce a small number of large, live young that are nourished by oophagy (Gilmore, 1993). In this unusual form of embryonic development, the pregnant female ovulates an enormous number of ova which are consumed by the embryos in the uteri. The embryos develop grossly swollen abdomens as they store large quantities of yolk for later growth. Oophagy was first described in porbeagles (Swenander, 1906, 1907; Shann, 1911, 1923) and salmon sharks (Lohberger, 1910), but has only recently been confirmed in shortfin and longfin makos (*Isurus oxyrinchus* and *I. paucus*) and white sharks (*Carcharodon carcharias*) (Gilmore, 1983; Stevens, 1983; Francis, 1996; Uchida et al., 1996).

The unusual bloated appearance of porbeagle embryos has led to a number of reports in the literature (Bigelow and Schroeder, 1948; Graham, 1956; Templeman, 1963), but the absence of a series of embryos at different stages of gestation has hampered attempts to understand their development. Litters usually consist of four embryos (Templeman, 1963; Gauld, 1989), which are thought to be born at about 60–80 cm total length (TL) (Shann, 1923; Compagno, 1984; Last and Stevens, 1994). Female size at maturity is often cited as 152 cm TL (Bigelow and Schroeder, 1948; Compagno, 1984; Last and Stevens, 1994), apparently based on two pregnant females reported to have been "about five feet long" (Shann, 1911). However, no other mature females under 2 m TL have been reported, leading some authors to regard the length at maturity as 2–2.5 m TL (Aasen, 1963; Pratt and Casey, 1990). The length of the gestation period is unknown; estimates, however, range from eight months to two years (Shann, 1923; Aasen, 1963; Gauld, 1989). The timing of parturition is variously stated as spring, summer, or autumn in the North Atlantic (Bige-

low and Schroeder, 1948; Aasen, 1963; Gauld, 1989). Thus, despite the early discovery of oophagy in porbeagles, little is known about their reproduction. Most parameter estimates are imprecise, and several are speculative or conflicting.

Few pregnant females have been reported from the Southern Hemisphere, and few details have been provided for any of them. Graham (1939, 1956) reported one caught at Otago Heads, New Zealand, in 1933. It had three embryos that were approaching full term and weighed 3.4–4.3 kg each. Graham (1956) also examined several other pregnant females but he reported few details. Duhamel and Ozouf-Costaz (1982) found four small embryos in a female caught in 1981 near Kerguelen Island in the southern Indian Ocean (51°S, 70°E).

Growth curves are available for northwest Atlantic porbeagles, based on modal analysis of length-frequency distributions, and back-calculation of length-at-age from bands on a vertebra (Aasen, 1963). They suggest that growth is relatively fast, at least in the first few years, and that longevity is 20–30 years. No growth information is available for the Southern Hemisphere.

Porbeagles have been exploited for their flesh for many decades, and have proven to be vulnerable to overfishing. A target longline fishery in the northwest Atlantic in the 1960s lasted only six years before collapsing (Anderson, 1990; Pratt and Casey, 1990). In the Southern Hemisphere, porbeagles have not been targeted, but they are frequently taken as bycatch in tuna fisheries, especially the pelagic driftnet fishery for albacore (*Thunnus alalunga*) during 1982–91 in the South Pacific (Murray, 1994; Yatsu, 1995), and the longline fishery for southern bluefin tuna (*Thunnus maccoyii*) and bigeye tuna (*Thunnus obesus*) in the southern Indian and Pacific Oceans (Stevens et al., 1983; Francis et al., 1999). In the New Zealand longline fishery, porbeagles are the second most commonly caught shark after the blue shark (*Prionace glauca*) (Francis et al., 1999).

The collapse of the northwest Atlantic fishery in the 1960s provides ample justification for a cautious approach to managing porbeagles. In view of recent increased landings in the North Atlantic (O'Boyle et al., 1996), and the size and scope of the tuna longline fishery in the southern oceans, there is an urgent need for improved information on reproduction, growth, and stock productivity as a basis for effective management. Much of the Southern Hemisphere longline fishery occurs in international waters, making monitoring and management difficult. Recently, the New Zealand and Australian governments implemented scientific observer programs to monitor catches of foreign and domestic longline vessels in their respective Exclusive Economic Zones (EEZs). These programs provided an opportunity to collect information on the reproduction and growth of porbeagles. In this paper, we describe the geographical distribution and length composition of sharks taken by longline vessels in the southwest Pacific, estimate the growth rate of embryos and juveniles, and describe embryonic development and oophagy. We also estimate the length of the gestation period, the timing of parturition, and the size at birth, and compare these with estimates for North Atlantic porbeagles.

Materials and methods

Data sources

Most of our data and specimens were collected by scientific observers aboard Japanese and domestic tuna longline vessels operating in the New Zealand and Australian EEZs. In New Zealand, fishing and observer effort was concentrated in two regions: 1) northeast New Zealand (north and east coasts of North Island and the Kermadec Islands), and 2) southwest New Zealand (east and west coasts of South Island) (Fig. 1). In Australia, most effort was around Tasmania (Fig. 2). New Zealand observers began recording the quantity of bycatch in 1987, measuring and sexing porbeagles in 1990, and examining females for embryos in 1992. In Australia, the respective years were 1988, 1990 and 1991. The primary task of observers was to monitor the target tuna species (mainly southern bluefin and bigeye tuna). Porbeagles were counted on most longline sets but were not always measured or examined for embryos. Therefore our data represent a subsample of the catch taken by observed longliners. The opportunistic nature of this collection process, and the low catch rate of pregnant females, necessitated the accumulation of specimens and data over a lengthy period.

Embryos collected by observers were supplemented by specimens and data from other sources, and the literature, including three litters from Heard and Kerguelen Islands in the southern Indian Ocean (Table 1). The four embryos from the Kerguelen female were deposited in the Museum National d'Histoire Naturelle (MNHN 1981-1432–1981-1435) (Duhamel and Ozouf-Costaz, 1982), and were photographed and remeasured for us by Duhamel.[1] A 185-cm-fork length (FL) female from Macquarie Island (Fig. 1) was the only intact pregnant female we examined.

[1] Duhamel, G. 1997. Museum National d'Histoire Naturelle (MNHN), 75231 Paris cedex 05, France. Personal commun.

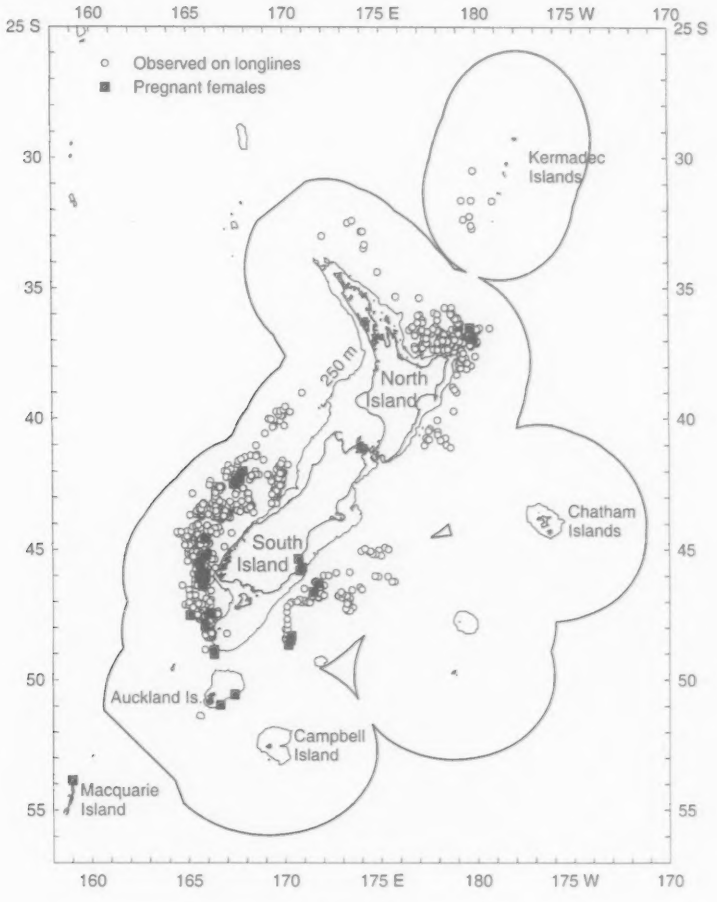

Figure 1

Map of the New Zealand region showing start positions of tuna longline sets from which porbeagles were recorded, and capture locations of pregnant females (n=35). The 250-m isobath and Exclusive Economic Zone are also shown.

Size and growth

Porbeagles were measured in one or more of three ways: precaudal length (PL; snout to the precaudal pit), FL (snout to the fork in the tail), and TL (snout to the tip of the tail). TL can be measured in two different ways—with the tail in the natural position (TL_{nat}) (Bigelow and Schroeder, 1948), or with the tail flexed down so that the upper caudal lobe lies parallel to the long axis of the body (TL_{flex}) (Compagno, 1984). Observers probably measured TL_{nat} on postnatal porbeagles because TL_{flex} is difficult to measure in species with a relatively rigid caudal fin. TL_{nat} measurements in embryos are not strictly comparable with TL_{nat} measurements in postnatal porbeagles because of the curved and folded nature of the caudal fin in embryos.

Most observers measured FL; therefore we adopted that as our standard. Regression equations relating FL to TL and PL are given in the "Results" section. Literature reports of TL were converted to FL before comparison with our data. Hereafter, FL is reported unless otherwise stated. Porbeagles were also sexed, weighed whole, and sometimes weighed after processing. Data were inspected for outliers on bivariate plots of PL, FL, TL, whole weight, and processed weight. Obvious errors were corrected if possible, and deleted if not. Before 1993, some New Zealand observers confused porbeagles and shortfin makos. We therefore restricted our New Zealand analyses of length, weight and location to data collected from 1993 onwards.

Initial inspection of the length-frequency data revealed modes that might correspond with juvenile age

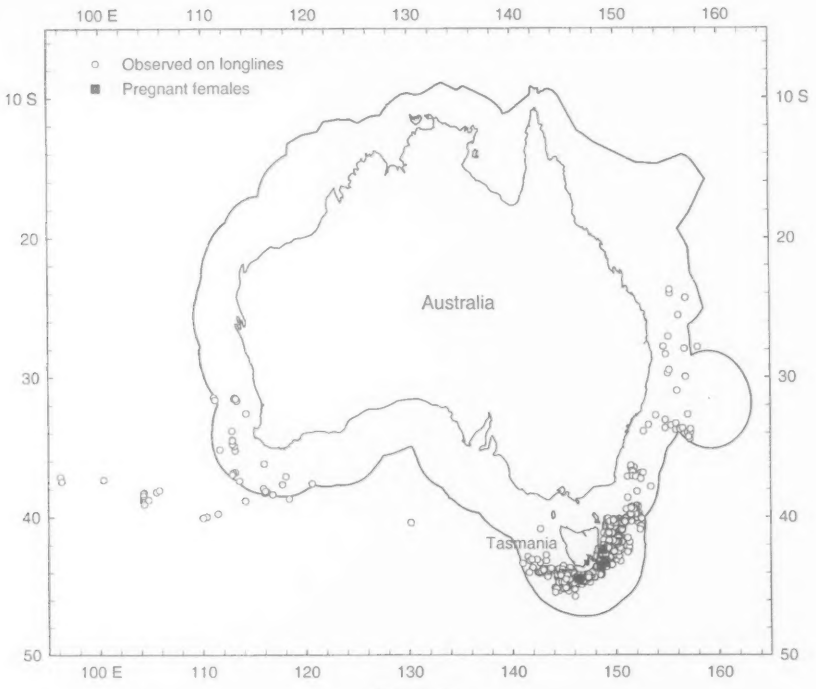

Figure 2

Map of the Australian region showing start positions of tuna longline sets from which porbeagles were recorded, and capture locations of pregnant females (n=5). The Exclusive Economic Zone is also shown.

classes. To assist modal discrimination, we limited our length-frequency analyses to the period April–July, during which 87% of New Zealand and 86% of Australian length measurements were taken. The MIX computer program (MacDonald and Pitcher, 1979; MacDonald, 1987; MacDonald and Green, 1988) was applied separately to the New Zealand and Australian length-frequency data for combined sexes to decompose the distributions into their component age classes. The program estimates the mean length, and the standard deviation of the lengths, for each age class, and the proportion of the sample in each age class. Length-frequency data were grouped into 3-cm class intervals, and truncated at 162 cm (New Zealand) and 150 cm (Australia) before analysis because large sharks were poorly represented in the samples. For each data set, we fitted a MIX model with three age classes and then progressively added extra age classes until there was no significant improvement in the χ^2 goodness of fit (MacDonald and Green, 1988). Occasionally, partially constrained fits (alternately fixing the standard deviations and proportions of one or two of the older age classes) were necessary for successful convergence (MacDonald and Green, 1988). This had no effect on the estimates of mean length, which were never constrained.

Embryos and ova

Embryos were placed in plastic bags and frozen; sometimes only partial litters were retained. Rarely, the uteri were removed intact and frozen with the embryos still inside. In the laboratory, embryos were thawed, sexed, and their jaws were examined for functional teeth. The embryos were then weighed, and measured (usually PL, FL, TL_{nat}, and TL_{flex}). FL was estimated from TL for three embryos without FL measurements (see "Results" section for regression equations). The liver and the contents of the stomach and intestine were weighed separately. Stomach contents were expressed as a percentage of total weight. Liver weight, and the weight of the intestinal contents were expressed as percentages of yolk-free embryonic weight to avoid distortions caused by the large variation in the stomach contents.

The uteri and right ovary[2] of the Macquarie Island female were examined. The diameters of a subsample of ovarian ova were measured using an image analysis system attached to a binocular microscope, and an

[2] The left ovary of lamnid sharks is vestigial (Pratt, 1988).

estimate was made of the total number present by counting ova in six weighed subsamples.

The four MNHN embryos from Kerguelen Island were measured for TL, FL, and weight in December 1997. Because there was a mean shrinkage of 4.4% from the original TL measurements (Duhamel and Ozouf-Costaz, 1982), we applied an equivalent shrinkage correction to the 1997 FL measurements. Fresh embryo weights were not reported by Duhamel and Ozouf-Costaz (1982), and we have not used the 1997 weights because they probably underestimate the original weights due to dissolution and leaching of lipids from the yolk in the stomach. Similarly, measurements from the Museum of New Zealand (NMNZ) embryos are not included here because of likely shrinkage and weight loss following preservation.

North Atlantic embryo lengths and dates of capture were obtained from the literature for comparison with Southern Hemisphere data (Swenander, 1906, 1907; Shann, 1911, 1923; Nordgård, 1926; Bigelow and Schroeder, 1948; Templeman, 1963; Gauld, 1989; Moss[3]; Newton[4]). For some litters, only one or two embryos were measured. Data were used only if they specified the month of capture, and some measurements that were known or thought to have been made on preserved specimens were corrected for shrinkage.

Results

Geographical distribution

Porbeagles have a wide latitudinal distribution. In the New Zealand region, they range from the Kermadec Islands (30°30'S) to Macquarie Island (53°52'S) (Fig. 1). In the Australian EEZ, they range from near the Tropic of Capricorn in southern Queensland (23°44'S) to south of Tasmania (45°44'S) (Fig. 2). The large number of capture records from northeast and southwest New Zealand, and around Tasmania, reflect concentration of fishing effort, and not necessarily high shark densities. Porbeagles also occur near Heard Island (51–52°S), and Kerguelen Island (51°S) in the southern Indian Ocean (Duhamel and Ozouf-Costaz, 1982).

Porbeagles were caught off southern Queensland (Fig. 2, north of 31°S) only in winter (June–September), when water temperatures were lowest. Sea surface temperature (SST) at the time of capture of six sharks off Queensland in July–August 1997 was 21.3–21.6°C, about 4°C lower than normal.

Sea surface temperature was recorded at about hourly intervals during hauling of each longline in New Zealand. The number of sharks caught per 1000 hooks (CPUE) was determined for each set and plotted against the mean of the hourly SSTs. There was no apparent trend in CPUE between 9.85°C (the minimum set temperature) and 19.5°C (mean CPUE=1.82, maximum=44.8, n=1292 sets). Between 19.5 and 23.0°C, mean CPUE was lower (mean=0.54, maximum = 5.0, n=105), and above 23.0°C no porbeagles were caught (n=23).

Most pregnant females were caught in the cooler southern waters of New Zealand and Australia (Figs. 1 and 2), and some were taken from the subantarctic Auckland, Macquarie, Heard, and Kerguelen Islands (50–54°S). However, two were also caught in northeast New Zealand. For longline-caught females, SST was 10.2–17.2°C (mean 12.9°C, n=32), and bottom depth at the capture locality was 600–4300 m (mean=2104 m, n=11). The two Heard Island pregnant females were taken by bottom trawl at depths of 248 and 259 m and bottom temperatures of 2.9 and 2.5°C, respectively. The Auckland Islands female was caught by midwater trawl at 160–164 m and a temperature of 11.9°C. Porbeagles have also been caught by bottom trawl near Macquarie Island at temperatures of 1°C (Williams[5]).

Length, weight, and growth

The relationships between PL and FL (both in cm) for New Zealand porbeagles were as follows:

$PL = -1.366 + 0.907\ FL$ (n=866, r^2=0.995,
$FL = 1.990 + 1.098\ PL$ range 61–223 cm FL, 54–208 cm PL

The relationships between TL and FL (both in cm) for Australian porbeagles were as follows:

$TL = 4.165 + 1.098\ FL$ (n=173, r^2=0.967,
$FL = -0.567 + 0.881\ TL$ range 63–180 cm FL, 71–202 cm TL)

Length-weight data were available for 641 New Zealand porbeagles (330 males, 309 females, and 2 unsexed) over the range 61–228 cm FL and 3–153 kg weight. However 96.7% of the sample was less than 150 cm FL; therefore the results represent only juveniles. There was no evidence from the raw data, or the residuals from a log-log regression, of a difference between the sexes. The regression equation for combined sexes was as follows:

[3] Moss, S. A. 1995. University of Massachusetts, North Dartmouth, MA 02747, USA. Personal commun.

[4] Newton, A. 1996. The Marine Laboratory, P.O. Box 101, Aberdeen, Scotland. Personal commun.

[5] Williams, R. 1997. Australian Antarctic Division, Tasmania, Australia. Personal commun.

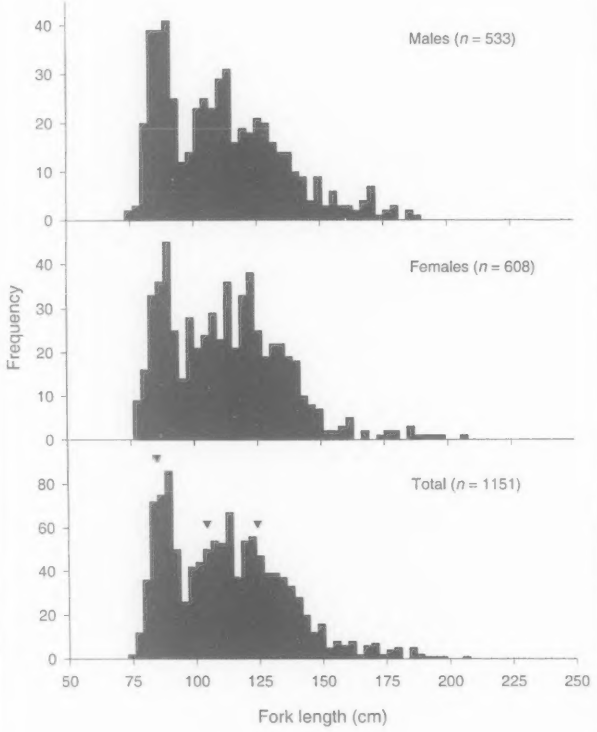

Figure 3A

Porbeagle length-frequency distributions by sex for April–July: (**A**) southwest New Zealand; (**B**) northeast New Zealand; (**C**) Australia. The bottom panel in each series includes some unsexed sharks. n = sample size. Triangles indicate mean lengths of the age classes identified by MIX modal decomposition.

$$\text{Log}_{10}(weight) = -5.050 + 3.128\ \text{Log}_{10}(FL), \quad (n = 641, r^2 = 0.956)$$

where weight is expressed in kg and FL in cm.

In New Zealand, length ranges were 64–228 cm for males and 61–206 cm for females (Fig. 3, A and B). Most were shorter than 150 cm. The size distributions were similar for males and females up to 150 cm, and the sex ratio (both subregions combined) was not significantly different from one (M:F=0.93:1; χ^2=1.99, P>0.1). However, males outnumbered females above 150 cm by 3.18:1 (χ^2=16.28, P<0.01).

In southwest New Zealand, there was a strong modal peak at 79–93 cm for both sexes, and for males there were also clear peaks at 100–115 cm and 118–133 cm (Fig. 3A). The best MIX fit to the combined sexes data consisted of three age classes whose mean lengths are shown in Fig. 3A. Sample sizes were small in northeast New Zealand; therefore no MIX model was applied. A strong mode was present at 67–88 cm (Fig. 3B), and indistinct modes were present for both sexes at about the same position as the second and third modes in southwest New Zealand.

In Australia, length ranges were 61–204 cm for males and 58–208 cm for females (Fig. 3C). Most were shorter than 150 cm, with a strong mode at 76–94 cm. The size distributions of males and females were similar. The sex ratio of sharks smaller than 150 cm did not differ significantly from one (1.07:1; χ^2=1.47, P>0.1), but males outnumbered females above 150 cm by 2.71:1 (χ^2=11.08, P<0.01). The best MIX fit to the combined sexes data consisted of five age classes whose mean lengths are shown in Figure 3C.

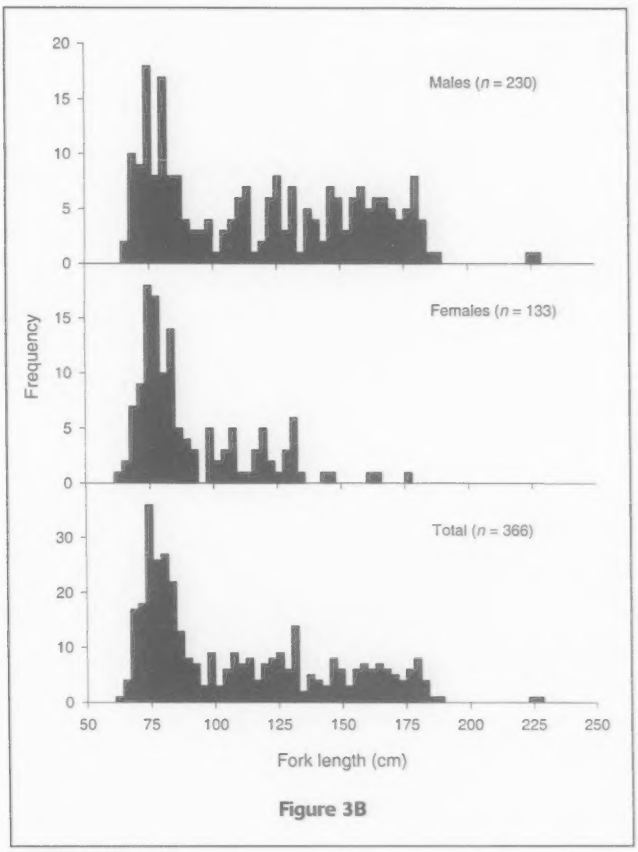

Figure 3B

The mean lengths of the modes for southwest New Zealand and Australia were plotted against age, which was calculated from a theoretical birth date of 1 June (see below) and mean sampling dates of 5 May and 16 June, respectively (Fig. 4). Thus the ages classes were sampled near their respective birth dates. We interpret the five Australian modes as representing sharks that were recently born, and 1–4 years old, and the three southwest New Zealand modes as those representing sharks that were 1–3 years old. The first length mode in the northeast New Zealand distribution was substantially shorter than the first southwest New Zealand mode and the second Australian mode, and we are uncertain about assigning an age to it. At age 1 year, southwest New Zealand and Australian porbeagles were similar in length, but for older ages New Zealand sharks were slightly larger. Growth in both regions was linear over the range of the data (Fig. 4):

southwest
New Zealand: $FL = 66.5 + 19.8\,(Age)$ (r^2=1.000)

Australia: $FL = 65.4 + 16.1\,(Age)$ (r^2=0.997)

Female length at maturity and reproductive development

Maturity status was not recorded; therefore we cannot estimated length at maturity. However, 37 pregnant females ranged from 167[6]–199 cm (mean=185 cm), suggesting that female maturity is reached around 165–180 cm.

The Macquarie Island female had four embryos 21.5–23.2 cm long. The right ovary was of the "internal" type, which is typical of lamnid sharks (Pratt, 1988). It weighed 2.75 kg (2.35% of total weight) and was undergoing active oogenesis. The entire ovary was packed with ova; other than a thin external envelope it had no macroscopically visible ovarian tissue. There was a single large efferent pore in the ovary, from which ova are shed (Stevens, 1983; Gilmore, 1993).

[6] FL of 167 cm was calculated from a PL of 151 cm.

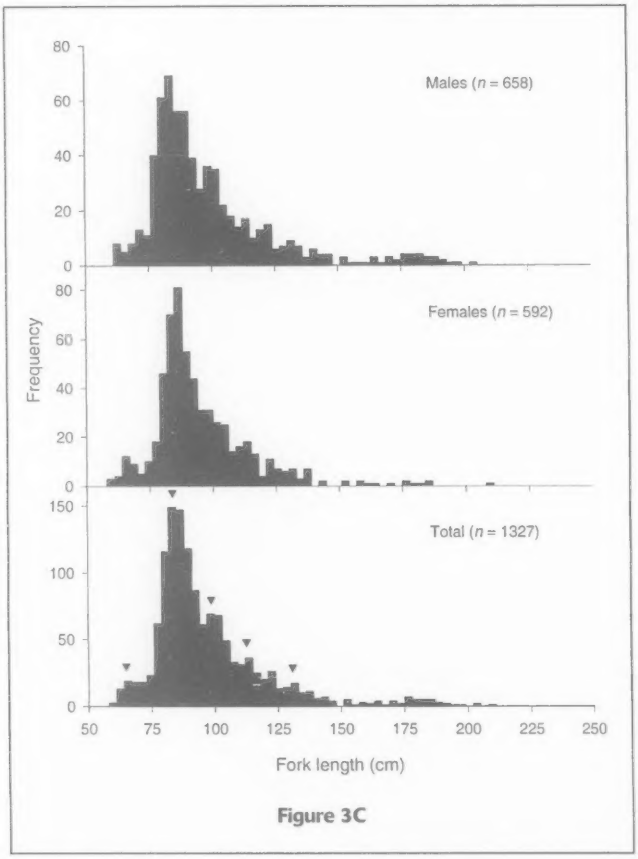

Figure 3C

The mean ovum count from six weighed subsamples was 72.2 ova per gram (SE=2.6), producing an estimated total number of 198,000 ova. Ova diameters were mostly 1.5–3.3 mm; a group of larger ova had diameters of 3.4–4.7 mm (Fig. 5). In addition to the embryos, the right uterus contained three egg capsules, and in the left uterus a 22.0-cm embryo had an egg capsule lodged in its mouth. Three of the capsules were empty and the fourth contained four ova (Fig. 6). Uterus width, estimated from a photograph containing a ruler, was about 10 cm. In other females with near-term embryos, uterus width was about 20 cm. The anterior quarter of the uterus had many longitudinal folds or pleats, and the rest was covered with small papillae and had a velvety texture.

Litter size, embryonic growth, and gestation

Data were obtained from 43 litters and 138 embryos (Table 1). All but four of the 40 litters for which litter size was known contained four embryos, two in each uterus. The exceptions were two litters reported by Graham (1939, 1956) with two and three embryos respectively, one reported by Hanchet [7] with three embryos, and a New Zealand longline-caught litter with two midterm embryos. Mean litter size was 3.85. Of 132 embryos that were sexed, 73 were males and 59 were females, producing a sex ratio not significantly different from one (χ^2=1.48, P>0.1).

Regression equations relating different embryo length measurements (in cm) were:

$PL = -0.125 + 0.885\ FL$ (n=97, r^2=0.999)
$TL_{nat} = 0.180 + 1.162\ FL$ (n=96, r^2=0.998)
$FL = -0.085 + 0.859\ TL_{nat}$ (n=96, r^2=0.998)
$TL_{flex} = 0.836 + 1.170\ FL$ (n=87, r^2=0.998)
$FL = -0.644 + 0.853\ TL_{flex}$ (n=87, r^2=0.998)

A log-log regression of yolk-free embryo weight (kg) against FL (cm) gave

[7] Hanchet, S. 1996. National Institute of Water and Atmospheric Research (NIWA), P. O. Box 893, Nelson, New Zealand. Personal commun.

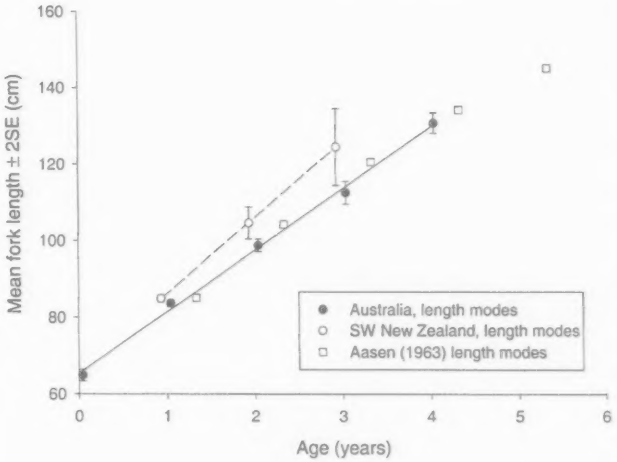

Figure 4

Porbeagle mean lengths-at-age determined by MIX analysis of the southwest New Zealand and Australian data in Figure 3, A and C, with fitted linear regressions. Also shown are Aasen's (1963) northwest Atlantic length-frequency modes, which were plotted assuming a 9 April theoretical birth date (1.7 months earlier than the Southern Hemisphere) and a mean sampling date of 9 August.

Table 1

Sources, numbers, collection periods and collection localities of Southern Hemisphere porbeagle embryos. NZ = New Zealand; NMNZ = Museum of New Zealand. Additional pregnant females reported by Graham (1956) were not included because of lack of data.

Source	Number of litters	Number of embryos	Collection period	Collection locality
Tuna longline	27	87	1992–98	New Zealand
Tuna longline	5	17	1991–95	Tasmania, Australia
Bottom trawl	2	8	1997	Heard Island
Bottom trawl	1	4	1995	Macquarie Island
Midwater trawl	1	4	1996	Auckland Island, NZ
NMNZ P22122	1	3	1987	Auckland Island, NZ
Hanchet[1]	2	2	1982–83	Otago, NZ
Berquist[2]	1	4	1998	Otago, NZ
Graham (1939, 1956)	2	5	1933	Otago, NZ
Duhamel and Ozouf-Costaz (1982)	1	4	1981	Kerguelen Island
Total	43	138		

[1] See Footnote 7 in the main text.
[2] Berquist, R. 1997. Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand. Personal commun.

$$\text{Log}_{10}(weight) = -4.719 + 2.916\ \text{Log}_{10}(FL) \quad (n=100,\ r^2=0.974)$$

The Kerguelen Island embryos reported by Duhamel and Ozouf-Costaz (1982) were the smallest in our sample, with estimated FLs of 9.6–10.4 cm. Our largest embryos were in litters of 62.0–67.0 cm, 64.2–65.6 cm, and 64.3–66.6 cm. Typically, all embryos in a litter were similar in length, but two litters each contained one unusually small embryo (runt) (Table 2). Variation in length within a litter increased with mean length, but the percentage range in length was relatively constant.

Table 2

Fork lengths and weights of stomach contents, intestinal contents, and livers of embryos from two litters that contained one unusually small embryo.

	Litter 1	Litter 2
Embryo fork lengths (cm)	22.5, 27.7, 29.0, 30.8	51.4, 55.3, 55.3, 63.8
Length range (cm)	8.3	12.4
Length range as percentage of mean length (%)	30.2	22.0
Smallest embryo		
Stomach contents (kg)	0.015	0.077
Intestine contents (kg)	0.007	0.069
Liver (kg)	0.003	0.056
Other three embryos		
Stomach contents (kg)	0.117–0.440	0.183–0.340
Intestine contents (kg)	0.114–0.296	0.092–0.130
Liver (kg)	0.006–0.017	0.151–0.364

All embryos were collected between 11 March and 16 July, reflecting the seasonality of the longline fisheries. The mean length of Southern Hemisphere embryos in a litter increased significantly ($P<0.01$) with sampling date (Fig. 7):

$$\textit{Mean FL}\ (\text{cm}) = 9.36 + 7.48\ \textit{month}, \quad (n=39, r^2=0.27)$$

where "month" is defined as the number of months elapsed since 1 January. There was considerable unexplained variability. For example, in April, mean embryo length varied between 20.3 and 63.5 cm. Inspection of the data by year of collection and sampling location showed that the variability was not caused by interannual or spatial differences. A regression equation fitted to North Atlantic embryo data (Fig. 7) was

$$\textit{Mean FL}\ (\text{cm}) = 23.51 + 6.78\ \textit{month} \quad (n=29, r^2=0.70)$$

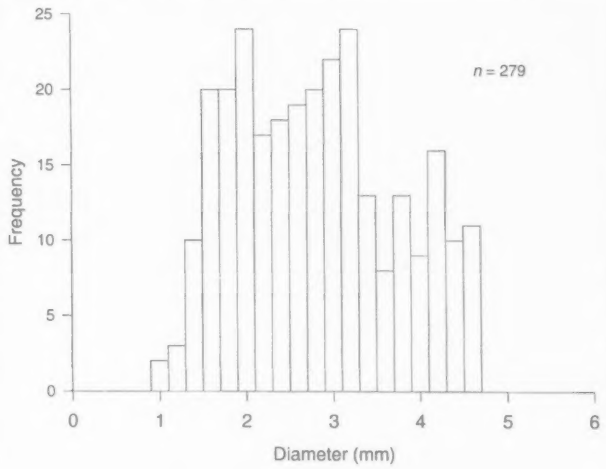

Figure 5

Frequency distribution of ovarian ova diameters from a pregnant porbeagle from Macquarie Island. n = sample size.

The North Atlantic regression explained a much higher proportion of the variation but displayed considerable length variability in early gestation. A homogeneity of slopes test showed that the regression slopes for the two hemispheres were not significantly different ($P=0.76$). The pooled data had a regression slope (=embryonic growth rate) of 7.1 cm per month, and the regression intercepts differed significantly (analysis of covariance, $P=0.004$) by 12.0 cm, which is equivalent to a temporal displacement of about 1.7 months.

Small, postnatal juveniles 58–68 cm long ($n=53$), were observed on Australian longlines between 22 May and 9 September, with a mean capture date of 15 July. Large embryos (up to 67 cm) were observed between mid-April and mid-June (Fig. 7). In the Southern Hemisphere, the large variation in embryo length at any one time, and the long period over which small juveniles, assumed to be newborn, were collected, indicate that the parturition period is lengthy. Parturition probably peaks in June–July (winter) but may

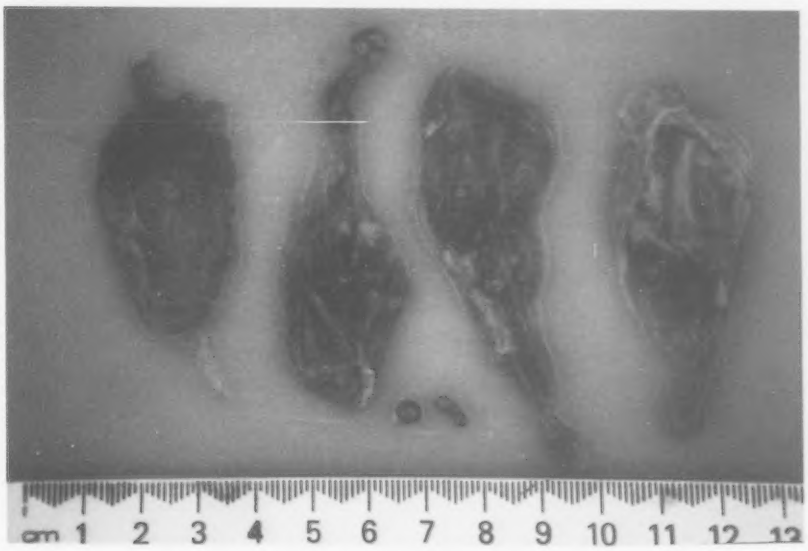

Figure 6

Four empty egg capsules found in the uteri and in the mouth of an embryo, from a pregnant porbeagle from Macquarie Island. Also shown are several loose ova found in one of the egg capsules. The largest egg case was 75 mm long.

extend from April to September. For aging purposes, we defined the theoretical birth date as 1 June. Based on the lengths of the smallest juvenile and the largest embryo, the length at birth is 58–67 cm. If a growth rate of 7.48 cm per month is maintained by Southern Hemisphere porbeagles throughout gestation, the gestation period is about 8–9 months. However, the unexplained variability in Fig. 7 compromises our ability to accurately estimate the gestation period.

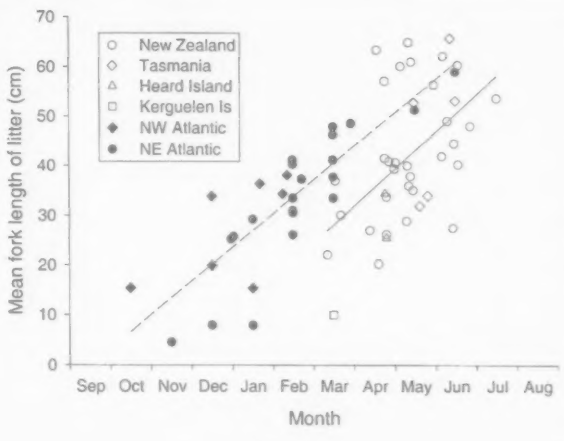

Figure 7

Monthly variation in the mean lengths of porbeagle embryos from four Southern Hemisphere locations (white symbols, n=39, r^2=0.27), and the northwest and northeast Atlantic (black symbols, n=29, r^2=0.70).

Embryonic development

Porbeagle embryos develop the distended yolk stomach that is characteristic of all oophagous lamnid sharks (Fig. 8). In the Kerguelen embryos (9.6–10.4 cm), such distension was already apparent. The caudal fin was notably curved, with the upper lobe much longer than the lower lobe, and there were no external gill filaments. At 19.8–20.7 cm, the bulging yolk stomach was the most noticeable feature, along with a marked lateral expansion of the head and branchial region (Fig. 8A). The body lacked pigmentation (except for the eyes), and appeared pink because of the presence of blood vessels under the skin. At 34.2 cm, the yolk stomach had become enormously distended, and measured 22.6 cm long by 15.9 cm high; the branchial and throat regions remained

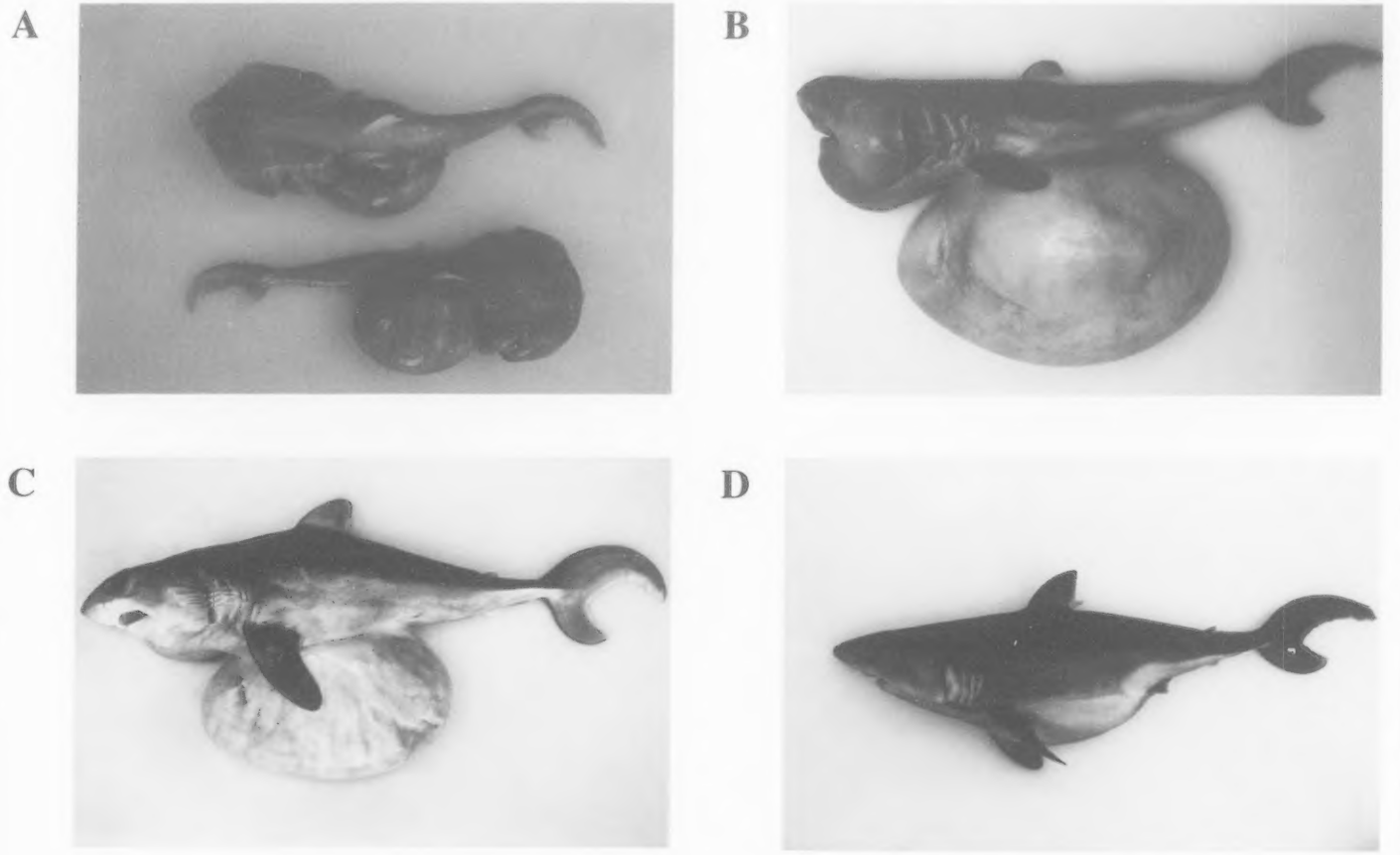

Figure 8

Changes in porbeagle embryo external shape with increasing size (not to same scale). (**A**) 20.7-cm female (top) and 19.8-cm male (bottom). (**B**) 34.2-cm female. (**C**) 40.3-cm female. (**D**) 58.0-cm female.

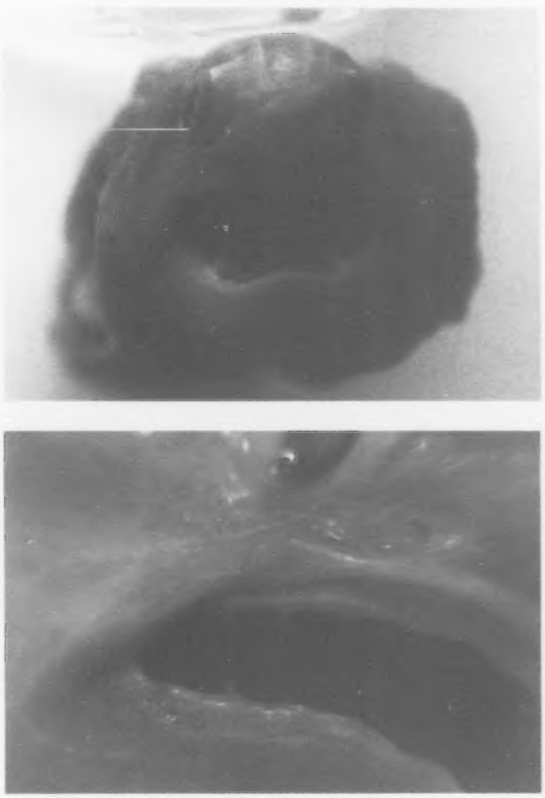

Figure 9

Anterior view of the heads of (**A**) 9.6-cm embryo, and (**B**) 26.4 cm female embryo showing the functional fangs. (Photo A. by G. Duhamel.)

swollen, and the upper body and pectoral fins had become pigmented (Fig. 8B). At 40.3 cm, pigmentation was essentially the same as in postnatal porbeagles, the swelling of the head had disappeared, and the yolk stomach had begun to shrink (Fig. 8C). At 58.0 cm the juvenile body form had been attained, apart from an enlarged abdomen (Fig. 8D). Other embryos around this size and larger had a more streamlined shape, with little noticeable abdominal distension.

Distension of the abdomen during early development causes the subdermal muscle layers to split along the ventral midline, extending anteriorly as far as the fifth gill slits. The expanding stomach protrudes between the muscle layers and stretches the abdominal skin. Later, the stomach shrinks back inside the muscle layers, and the stretched skin returns to its original shape. A distinct "scar" remains in the ventral midline in the area between the origins of the pectoral fins and the fifth gill slits, marking the anteriormost point of the split muscle layers.

Small embryos had large, erect, tubular, recurved "fangs" in both jaws (Fig. 9, A and B). These teeth, which were quite unlike those found in postnatal porbeagles, were clearly functional. In the Kerguelen embryos (9.6–10.4 cm), the tooth formula was (1+1/1+1), and the lower teeth were massive in relation to mouth size (Fig. 9A). Larger embryos (19.8–38.3 cm) had more functional upper teeth (3+3/1+1) (Fig. 9B). Additional minute teeth were visible under a light microscope, but they appeared vestigial and nonfunctional and were not included in the tooth formula. Replacement fangs were present behind the functional series, but they were irregularly spaced and usually located between the functional teeth. Oval scars on the gum of both jaws external to functional fangs indicated that fangs are progressively shed and replaced. The largest

embryo with fangs was 38.3 cm, and the smallest embryo without fangs was 33.9 cm. In the range of overlap, there were 12 embryos with fangs and 8 without fangs. Therefore, the fangs are shed between 34 and 38 cm.

Most of the larger embryos without fangs possessed several series of developing, nonerect, nonfunctional teeth shaped like those found in postnatal porbeagles, except that they lacked lateral cusps. One near-term litter had three embryos with nonfunctional upper teeth but partially erect lower teeth, whereas the fourth had nonfunctional teeth in both jaws.

The stomach contents consisted wholly or mostly of viscous, amorphous, light yellow yolk. In many embryos there were also discrete masses of clear, white or greyish gelatinous material, probably the remains of empty egg capsules, embedded in the yolk. This gelatinous material usually represented less than 10% of the stomach contents and has been included in the yolk weights reported below. Occasionally we found shed fangs in the stomachs, but the thick glutinous nature of the yolk made them difficult to find, and it was impossible to assess their frequency or abundance.

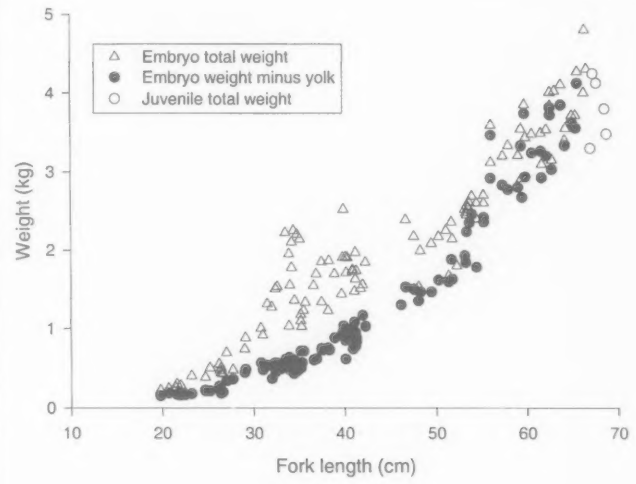

Figure 10

Variation in total weight and yolkfree weight with length for porbeagle embryos. Also shown are total weights of five small juveniles.

Embryonic total weight increased rapidly between 20 and 35 cm, changed little between 35 and 50 cm, then increased again during the rest of the gestation period (Fig. 10). Embryonic weight minus yolk weight increased steadily throughout gestation. The weights of five free-living juveniles shorter than 70 cm were similar to the yolk-free weights of the largest embryos.

The weight of yolk in the stomach increased steadily between 20 and 30 cm, before increasing rapidly to peak at 30–42 cm (Fig. 11). Yolk weight at 30–42 cm varied from 0.39 kg to 1.82 kg, representing 26.7–80.8% of total body weight. Absolute and percentage yolk weight both generally declined at lengths greater than 42 cm. Three embryos longer than 60 cm still had around 1 kg of yolk in their stomachs, but it represented a low proportion of their total weight (ca. 17–22%[8]). All embryos longer than 50 cm had yolk or gelatinous material in their stomachs, suggesting that the yolk may not be completely digested before birth.

The spiral valve of the intestine contained a thick, gritty, greenish-brown sludge that is the waste product of yolk digestion. The smallest embryos dissected (19.8–20.7 cm) contained small amounts of waste, and the quantity of waste increased steadily throughout gestation (Fig. 11). Intestinal contents composed the greatest percentage of yolk-free weight at 30–50 cm. Newborn porbeagles had smaller quantities of intestinal waste than large embryos, suggesting that the waste is retained until after birth.

Liver weight increased exponentially with FL, showing the most rapid increase above 55 cm (Fig. 11). Relative liver weight also increased to a maximum in the longest embryos. Postnatal porbeagles usually had smaller livers, both in absolute and relative terms, than those of the largest embryos, indicating that energy reserves stored in the liver are consumed by the young after birth.

The runts in two litters had low stomach and intestine contents, and liver weights (Table 2). The 22.5-cm runt in litter 1 had numerous short (1–3 mm) lacerations on its distended abdomen, and a few elsewhere on its body, presumably inflicted by the teeth of its larger sibling. However none of the gashes had penetrated the body cavity, and they did not appear to be life-threatening.

Five pairs of uteri containing embryos were obtained. The orientation of the embryos could be determined for only five of the 10 uteri; these five uteri contained embryos 38.2–62.6 cm long. In all cases, the two embryos were facing in opposite directions.

[8] Not shown in the percent yolk weight panel of Fig. 11 because embryo total weights were not measured accurately.

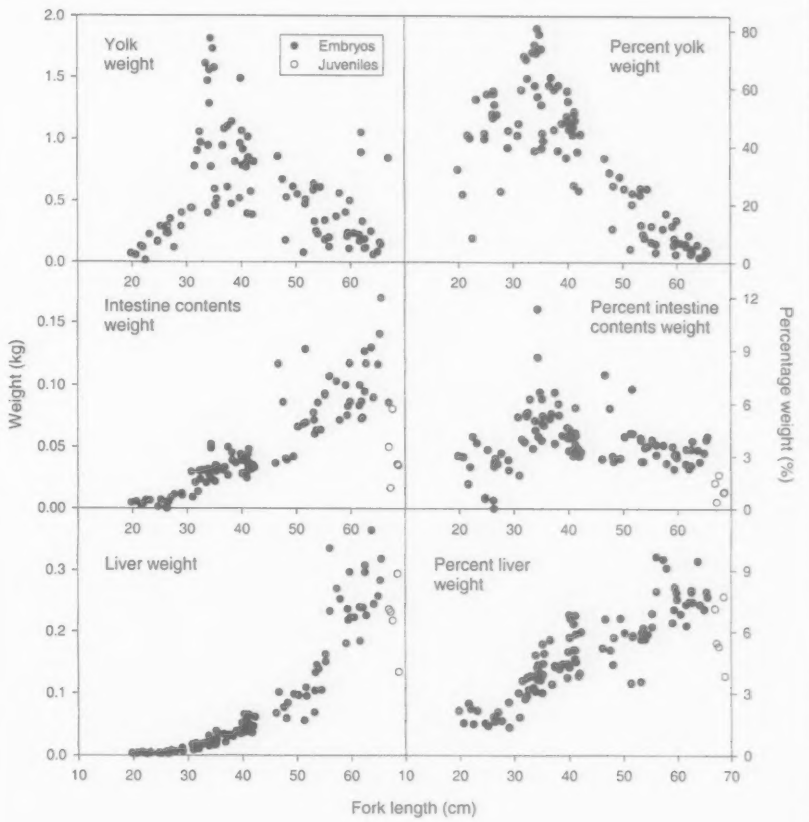

Figure 11

Variation in the weight of yolk, intestine contents, and liver with length (left panels), and the percentage of the total weight contributed by yolk, and percentages of yolkfree weight contributed by intestinal contents and liver (right panels) for porbeagle embryos. Comparable data for five small juveniles are also shown.

Discussion

Geographical distribution

Porbeagles occur throughout the New Zealand EEZ and the southern half of the Australian EEZ. On the east coast of Australia, they reach subtropical waters (to 23°44'S), but only during winter. At the other extreme, porbeagles are found in subantarctic waters in the Indian and southwest Pacific Oceans, reaching almost 54°S. Our results are consistent with observations of porbeagles between about 28 and 58°S across the entire South Pacific between New Zealand and Chile (Yatsu, 1995). Porbeagles are caught only north of 30°S in winter–spring (August–November), and they penetrate farther south during summer and autumn (Yatsu, 1995).

Sea surface temperature at locations where porbeagles were caught was 9.9–22.6°C, but catch rates were low above 19.5°C. This is similar to SSTs reported previously for porbeagles in the South Pacific by Stevens et al. (1983) (7.6–22.8°C, with most captures less than 16.7°C) and Yatsu (1995) (5–20°C with most captures less than 15 °C). Temperatures at the actual depth of capture would be similar to these because most longlines fished at 50–100 m depth, which is shallower than the depth of the mixed ocean layer in autumn–winter. Bottom temperatures for trawl-caught porbeagles were as low as 1–3°C, which is consistent with temperatures of 1.7°C (Svetlov, 1978) and 3.1–4.1°C (Templeman, 1963) reported elsewhere. Thus the temperature range inhabited by porbeagles in the Southern Hemisphere is probably about 1–23°C, with abun-

dance declining above about 19°C. The preferred temperature in the North Atlantic is less than 18°C (Aasen, 1963).

Porbeagles can maintain their body temperature up to 11°C above that of the surrounding water (Carey et al., 1985). Among the small group of endothermic sharks, they are exceeded in this capacity only by salmon sharks (Carey et al., 1985). The apparent preference for higher latitudes by large (Yatsu, 1995) and pregnant porbeagles may indicate an increased ability to thermoregulate in larger sharks. High body temperature is probably necessary for lamnid sharks to function as active predators of fast-moving prey in cold water (Goldman, 1997).

In the North Atlantic, porbeagle abundance varies seasonally and spatially (Aasen, 1961, 1963; Templeman, 1963; Mejuto and Garcés, 1984; Mejuto, 1985; Gauld, 1989), and there are indications of seasonal variability in their vertical distribution (Bigelow and Schroeder, 1948; Aasen, 1961, 1963). Limited tagging results show that they are capable of movements up to 2370 km (Aasen, 1962; Stevens, 1976, 1990). In combination with evidence of seasonal latitudinal migration in the South Pacific (Yatsu, 1995), this range of movement suggests that Southern Hemisphere porbeagles may exhibit complex seasonal, spatial, and length-related distribution patterns. Our data were collected mainly during April–July, and therefore provide little information on seasonality.

Length, weight, and growth

Several length-weight relationships have been published for the North Atlantic (Aasen, 1961; Mejuto and Garcés, 1984; Gauld, 1989; Stevens, 1990; Ellis and Shackley, 1995; Kohler et al., 1995). All were based on small samples, except those of Gauld (1989), who found a significant difference between males and females above 180 cm. Our New Zealand sample comprised mainly sharks less than 150 cm, and compared with the length-weight relationships of Mejuto and Garcés (1984), Gauld (1989) and Kohler et al. (1995), our length-weight relationship rises too steeply beyond 150 cm. Our relationship should therefore not be extrapolated beyond 150 cm.

The largest porbeagles in our samples were 228 cm (male) and 208 cm (female). The maximum length reached in the North Atlantic is not clear. Maxima of 253 cm (288 cm TL) and 278 cm (317 cm TL) for Scottish males and females, respectively, appear to be the largest reliable measurements (Gauld, 1989). Lengths of 294–325 cm ("estimated ... 11 feet", "ca 11–12 feet", or "340–370" cm TL) reported by McKenzie (1959) and Templeman (1963) were obviously not measured accurately and may have been overestimated. A TL of 12 feet equals 365.8 cm TL, which likely forms the basis for the maximum length of 365 cm TL reported by Pratt and Casey (1990). If Southern Hemisphere porbeagles grow as large as those in the North Atlantic, our samples do not include the larger size classes. Tuna longlines catch shortfin makos that exceed 300 cm FL (senior author, pers. obs.); therefore, they should be capable of retaining large porbeagles. The latter evidently inhabit latitudinal or depth ranges outside our sampling area, which is inhabited mainly by juveniles.

Males and females were equally represented at lengths up to 150 cm. At greater lengths, males significantly outnumbered females by about 3:1 in both New Zealand and Australia. Skewed sex ratios have been reported frequently in the North Atlantic, in favor of males (Mejuto and Garcés, 1984; Mejuto, 1985; Ellis and Shackley, 1995), females (Gauld, 1989), or either sex depending on the length range or sample (Aasen, 1963; O'Boyle et al., 1996). Aasen (1963) found that the overall sex ratio in large samples was close to 1:1. These results indicate that juveniles do not segregate by sex, but that larger sharks do.

MIX analysis discriminated 3 and 5 length modes respectively in southwest New Zealand and Australia, which we interpret as age classes. In northeast New Zealand, the mean sampling date was 25 June, over seven weeks after the mean sampling date for southwest New Zealand; therefore the first mode in the former could represent new-born sharks. Alternatively, it may represent slow-growing one-year-olds. The latter interpretation is supported by the similarity of the positions of modes 2 and 3 in both northeast and southwest New Zealand.

Juveniles grow linearly and rapidly, reaching 110–125 cm FL in three years. They may grow slightly faster in southwest New Zealand (20 cm/year) than in Australia (16 cm/year), but the standard errors were large for New Zealand 2- and 3-year-olds, and the comparison could be biased by incorrect determination of the number of modes in the length-frequency data. The modal lengths for Australian juveniles agree well with the first four modes for northwest Atlantic porbeagles presented by Aasen (1963) (Fig. 4), providing that our modes represent age classes. Aasen (1963) presented two growth curves—one based on length-frequency modes, and the other on back-calculated lengths-at-age from a single vertebra from a 226-cm female. [9] His two growth curves were practically identical.

Our growth estimates also agree with length-increment data for five tagged northeast Atlantic porbea-

[9] In a preliminary report, Aasen (1961) presented vertebral age data for 50 porbeagles. However the mean lengths-at-age differed substantially from those presented later, and Aasen (1963) stated that his earlier results "were not very accurate."

gles (Stevens, 1976, 1990). Their lengths at tagging and recapture were only estimated; therefore growth increments were approximate. The sharks were relatively small when released (84–105 cm), and were at liberty for 0.75–13 years. Annual growth increments were about 9–32 cm, with a mean of 20.4 cm. Despite good agreement among all sources of porbeagle growth data, a vertebral-based growth curve from adequate sample sizes and covering the full size range is still required for both hemispheres.

Longevity is unknown, but Aasen (1963) aged his 226 cm female as 19+ years and suggested that they may live around 30 years. This conclusion needs confirmation because longevity is often used to estimate the natural mortality rate, which is an important parameter in population models.

Length at maturity and reproductive development

The lengths of pregnant females (167–199 cm, mean 185 cm) suggest that females mature at about 165–180 cm. This estimate is consistent with reports of a 191-cm mature female from the South Atlantic (Svetlov, 1978), and immature females of 138, 139, and 150 cm from around New Zealand (Stevens et al., 1983; Duffy[10]). The length at maturity of North Atlantic females is controversial. Shann (1911) reported two pregnant females of "about five feet long" (152 cm TL, or 133 cm FL). In both cases, the length estimate was probably third-hand, and it is unlikely that the females were measured. That estimate of length at maturity, which we believe to be unreliable and too low, has permeated the literature (Bigelow and Schroeder, 1948; Compagno, 1984; Last and Stevens, 1994). Other authors have reported a wide maturity range of 175–220 cm FL (Aasen, 1961, 1963; Pratt and Casey, 1990). Templeman (1963) and Moss[3] reported females of 191 and 203 cm to be immature. The smallest mature females reported by Templeman (1963) and Gauld (1989) were 203 and 196 cm respectively, and Pratt (1993) examined one of 227 cm. Aasen (1961) showed that uterine width increased rapidly in females longer than 197 cm. These results suggest that North Atlantic females mature at about 195–205 cm (218–229 cm TL), which is higher than the range determined for the Southern Hemisphere. A similar between-hemisphere difference in length at maturity has been found for shortfin makos (Mollet et al.[11]).

There was no information on length at maturity in males in our data. On the basis of changes in clasper length and calcification, North Atlantic males apparently mature at a smaller size than do females, in the range 131–175 cm (150–200 cm TL) (Aasen, 1961; Ellis and Shackley, 1995).

The "internal" type ovary from our Macquarie Island pregnant female conformed with the morphology found in all lamnid and alopiid sharks examined so far (Pratt, 1988). It weighed 2.75 kg (2.35% of total weight). The ovary of Swenander's (1906, 1907) 246-cm North Atlantic pregnant female measured 41 by 28 cm, and weighed 6.3 kg, or 3.6% of estimated total weight. Mean embryo lengths in the two females were 22.1 cm and about 25 cm respectively. The embryo stomach contents peak at 30–42 cm, suggesting that ovarian size and ovulation may peak when embryos are about 25–30 cm long. In the shortfin mako, the ovary of an actively ovulating female with early-term embryos weighed about 5% of her total weight, whereas the ovaries of females carrying near-term embryos were spent and weighed as little as 0.2–0.3% of total weight (Mollet et al.[11]). In sandtiger sharks (*Carcharias taurus*), relative ovarian weight peaks at 6–7% of body weight, and then declines during the second half of gestation (Gilmore et al., 1983; Gilmore, 1993; Mollet et al.[11]). A low relative ovary weight was also reported in a longfin mako with near-term embryos (Gilmore, 1983).

The size-frequency distribution of ova from the Macquarie Island female indicated that they are ovulated at around 4–5 mm. Ova diameters measured in two pregnant North Atlantic females after preservation in 10% formalin were mainly in the range 2.3–4.3 mm, with the largest measuring 6.0 mm (Moss[3]). Swenander (1906, 1907) reported ova diameters between 1 and 5–6 mm in a North Atlantic pregnant female, and encapsulated 4–5 mm ova in the uteri of another female. Maximum ova diameters in other oophagous sharks range between 4 and 10 mm (Springer, 1948; Bass et al., 1975; Otake and Mizue, 1981; Gilmore, 1983; Gilmore et al., 1983; Stevens, 1983; Uchida et al., 1996; Chen et al., 1997).

Empty and near-empty egg capsules were found in the uteri of the Macquarie Island female, and in the mouth of one of its embryos. Moss[3] also found empty egg capsules in the mouths and gill slits of embryos measuring 33.8 and 36.2 cm. Apparently, embryos are capable of rupturing egg capsules and swallowing the contents, although the occasional presence of gelatinous material resembling egg capsules in embryo stomachs (Swenander, 1907; this study) suggests that whole or empty capsules are sometimes swallowed. Swenander (1906, 1907) found over 40 egg capsules, each measuring about 80 by 15 mm and containing

[10] Duffy, C. 1998. Department of Conservation, Private Bag 3072, Hamilton, New Zealand. Personal commun.

[11] Mollet, H. F., G. Cliff, H. L. Pratt, and J. D. Stevens. 1998. Reproductive biology of female shortfin mako *Isurus oxyrinchus* Rafinesque 1809. H. F. Mollet, Monterey Bay Aquarium, Monterey, California 93940, Unpubl. manuscript.

21–28 individual ova, in the uteri of a female that contained four embryos about 4.3–4.7 cm long. Duhamel and Ozouf-Costaz (1982) found 102 nonfertile eggs in the uteri of their Kerguelen Island female, which carried embryos of 9.6–10.4 mm. No photographs were taken of the eggs, and it is unclear whether they were egg capsules or individual ova (Duhamel[1]).

The emerging picture for oophagous sharks is that large numbers of nutritive egg capsules accumulate in the uteri during the early stages of gestation, and they are rapidly consumed as the embryos grow large enough to puncture and eventually swallow them (Gruber and Compagno, 1981; Otake and Mizue, 1981; Gilmore, 1983; Gilmore et al., 1983; Mollet et al.[11]). Ovulation peaks during midgestation, but full egg capsules are rarely found in the uteri, probably because they are eaten soon after entering the uteri. During later gestation, ovulation ceases and embryos metabolise their accumulated stomach contents for energy, growth, and storage in the liver.

Litter size, embryonic growth, and gestation

In 36 out of 40 of our pregnant females, litter size was four, with a mean of 3.85 embryos. Litters of two or three were occasionally recorded. In the North Atlantic, Shann (1911, 1923) stated that litters commonly consisted of two embryos (range 1–4), but his data almost certainly included several partial litters (see Table I in Shann, 1923). Templeman (1963) found three, four and four embryos in his three litters, and Gauld (1989) found four to be the most common number of embryos in a litter, with a mean of 3.7 (n=12).[12] One litter of five has also been reported (Bigelow and Schroeder, 1948). Our sample of Southern Hemisphere litters is the largest yet assembled, and Gauld's (1989) is the largest from the North Atlantic. Both samples had very similar mean numbers of embryos. We conclude that litter size is usually four, but smaller litters are occasionally found; litters larger than four are extremely rare. Some litters with fewer than four embryos were probably incomplete. Abortion of embryos during capture is common among nonlamnid sharks, but it is difficult to imagine midterm embryos with grossly distended abdomens being aborted, even when the mother is compressed in a trawl net. One of our New Zealand longline litters containing two midterm embryos, 37.5 and 38.3 cm long, was presumably complete. Abortion of near-term embryos is quite possible.

[12] Gilmore's Table 1 contains a number of errors. The reference to Aasen (1966) in the *Lamna nasus* section should presumably be Aasen (1963); the two Swenander (1907) litters contained four embryos each rather than 2; and the 219-cm TL shark with four embryos attributed to Nakaya (1971) was actually a male, and no embryos were mentioned by Nakaya.

Linear regressions fitted to the length-month data for both hemispheres suggest that embryos have a rapid growth rate of about 7 cm per month, but there was much unexplained variability (Fig. 7). The estimated growth rate is almost twice that of shortfin mako embryos (3.7 cm per month) (Mollet et al.[11]). The length of the gestation period appears to be about 8–9 months in both hemispheres. During July–September, Aasen (1963) found no embryos in the northwest Atlantic despite examining hundreds of mature females. He argued that the gestation period was 8 months, and that the females he examined were undergoing a short rest period between pregnancies. Our interpretation agrees with that of Aasen (1963).

A contrary hypothesis involving a gestation period of 1–2 years has been advanced by Shann (1923) and Gauld (1989). They argued that the high variability in embryo length and the apparent presence of two cohorts of embryos were inconsistent with a gestation period of less than one year. We cannot rule out their hypothesis, and we are conscious that our Southern Hemisphere data are limited in seasonal scope and that pooling data across locations and years is not desirable. However, we believe the data from both hemispheres are most consistent with a gestation period of less than one year. The implied rapid embryonic growth rate is not unreasonable given the abundant embryonic food supply and the relatively high growth rates of postnatal juveniles discussed above.

The high variability in the embryo length data might be explained by an extended mating period. In the northwest Atlantic, Aasen (1963) found males with seminal vesicles that were filling at the end of August, indicating that mating would begin in September. Pratt (1993) reported a mature female caught in October with moderate amounts of spermatozoa in the oviducal gland and with fresh vaginal abrasions. A mature male with haematose claspers was caught on the same longline, providing strong evidence of mating in October. Gauld (1989) found females with fresh bite marks, thought to be inflicted during mating, on females near the Shetland Islands in December–January. These observations suggest that mating lasts from September to January in the North Atlantic.

If our hypothesis of rapid embryonic growth and high intracohort variability is correct, parturition probably peaks in June–July (winter) in the Southern Hemisphere and possibly extends from April to September. Parturition in the Northern Hemisphere may peak around two months earlier (spring–summer). The presence of distinct length modes in juvenile length-frequency distributions from New Zealand, Australia, and the northwest Atlantic (Aasen, 1963) confirms that parturition is restricted to part of the year, rather than occurring year-round. Svetlov (1978) reported the capture

of a female in the South Atlantic in March and stated: "The shark had recently spawned, to judge by external characters (an inflamed area of the body around the anus) and the state of the ovaries." He did not elaborate further on the ovaries, so it is difficult to assess his report. If the female was postpartum, the timing is one month earlier than our suggested parturition period for Southern Hemisphere porbeagles. Another possibility is that the inflammation around the cloaca may have been the result of recent mating.

If the gestation and rest periods combined last one year, and females reproduce every year, annual fecundity is 3.85 young per female. If there is a resting period of just over one year between pregnancies, annual fecundity would be half that amount. It is therefore important to determine whether Aasen's (1963) suggestion of a one-year cycle is valid for both hemispheres. The gestation period in shortfin makos is thought to be 18 months, with a reproductive cycle of three years (Mollet et al.[11]). Interestingly, the greater average fecundity of makos would result in an annual fecundity of about four per female (Mollet et al.[11]), which is similar to the reproductive output of porbeagles assuming a one-year cycle.

For Southern Hemisphere porbeagles, the length at birth was estimated to be 58–67 cm (68–79 cm TL), based on the lengths of the largest embryos and the shortest postnatal juveniles. In the North Atlantic, the largest reported embryos were 60–64 cm (Pennant, in Shann, 1911) and 65 cm (Gauld, 1989). Bigelow and Schroeder (1948) reported a 55.7-cm specimen (66.0 cm TL; USNM 47528), but it was an embryo rather than a postnatal juvenile (Williams[13]). The embryo would have been about 58 cm before preservation. Postnatal porbeagles of 66 and 70 cm were reported by Imms and Day respectively (in Shann, 1911). These observations indicate that porbeagles are born at about the same length in both hemispheres.

Embryonic development

We assembled a comprehensive series of porbeagle embryos ranging from early gestation (9.6 cm long) to full-term, enabling us to describe the main morphological changes that occur during gestation. Previous studies that described and illustrated embryos were based on only a few embryos, most of which were midterm (Swenander, 1906, 1907; Shann, 1911; Nordgård, 1926; Bigelow and Schroeder, 1948; Templeman, 1963).The following review of embryonic development is derived from our observations, supplemented by literature reports.

[13] Williams, J. T. 1997. National Museum of Natural History, Washington DC 20560–0159. Personal commun.

Embryos 4.3–4.7 cm long have external gills and well-developed branchial regions (Swenander, 1907). They have nearly absorbed their yolk sacs and have a large spiral valve, but there is no yolk in the digestive system. Swenander (1907) stated that "these embryos are too small to be able to swallow entire egg capsules and their teeth are not sufficiently developed to tear open the egg capsules." They have not begun to feed at this stage, despite the large number of egg capsules present in the uteri, but precocial teeth have already formed. At 10 cm, the lower jaw contains two relatively massive fangs (Fig. 9A) that appear capable of tearing open egg capsules. The upper teeth are much less developed and there is only one functional tooth on each side of each jaw. Lower jaw teeth also develop earlier than upper jaw teeth in sandtiger sharks (Hamlett, 1983). The abdomen is swollen and the embryos have presumably begun feeding (the stomachs were not dissected). Large numbers of egg capsules are present in the uteri. The external gills have been resorbed. By 15 cm, the abdomen is distended, and the head and branchial region are gelatinous and grossly enlarged (Bigelow and Schroeder, 1948).

Between 20 and 42 cm, development is dominated by the consumption of large numbers of egg capsules, leading to a great increase in the relative size and distension of the yolk stomach (Swenander, 1907; Shann, 1911, 1923; Bigelow and Schroeder, 1948; Templeman, 1963; Moss[3]; Fig. 8). Large fangs are present in both jaws (Swenander, 1907; Templeman, 1963; Moss[3]; Fig. 9) and are used to open the egg capsules before removal of the contents; how this is accomplished is unknown. At 30–42 cm, yolk accounts for up to 81% of total weight (Fig. 11; Templeman, 1963). The stomach yolk in midterm embryos of shortfin makos peaks at about 60–70% of total weight (Mollet et al.[11]). Relative and absolute amounts of yolk in porbeagles and other oophagous sharks decline during the rest of gestation (Fig. 11; Mollet et al.[11]).

Porbeagle embryos shed their fangs at about 34–38 cm. Embryonic fangs (the "emb" teeth of Gilmore, 1993) have also been reported in salmon sharks, common and bigeye thresher sharks (*Alopias vulpinus* and *A. superciliosus*), shortfin makos and sandtiger sharks (Lohberger, 1910; Bass et al., 1975; Gruber and Compagno, 1981; Gilmore et al., 1983; Hamlett, 1983; Gilmore, 1993; Chen et al., 1997). They probably occur in all oophagous species, and in at least some species, they are shed part-way through gestation. In bigeye thresher sharks, fangs appear at about 11 cm TL and disappear at about 60 cm TL (Chen et al., 1997). In sandtiger sharks, they appear at 4–5 cm TL, and are lost some time before birth (Gilmore et al., 1983; Hamlett, 1983).

We suspect that female porbeagles cease ovulation at about the time the embryonic fangs are lost, as

has been reported for shortfin makos and sandtigers (Gilmore et al., 1983; Mollet et al.[11]). The embryos then rely on the yolk stored in their stomachs to provide the energy needed for growth and respiration during the rest of the gestation period. However it is also possible that females continue ovulating, and that the toothless embryos feed by swallowing whole egg capsules or by squashing them in their mouths. Whole egg capsules have been reported from the stomachs of near-term embryos of the bigeye thresher shark (Gilmore, 1983; Moreno and Morón, 1992), but not from near-term lamnid sharks. Clarification of this point requires examination of the ovaries of near-term females to assess their ovulatory state.

Above 35 cm, the waste products of yolk digestion continue to accumulate in the intestine. The greenish coloured waste is characteristic of oophagous sharks (Swenander, 1907; Lohberger, 1910; Shann, 1923; Springer, 1948; Uchida et al., 1996). The gritty nature of the intestinal contents was also mentioned by Swenander (1907) and has been reported to consist of "crystal-like pieces" in white shark embryos (Uchida et al., 1996). The composition of this material is unknown. The liver grows most rapidly in the second half of gestation as energy reserves are transferred to it for storage. An increase in the relative weight of the liver in larger embryos has also been observed in shortfin makos (Mollet et al.[11]).

During the second half of gestation, several series of "post-natal" teeth develop, but they are folded back in the jaws and are nonfunctional. In white sharks, some of these teeth are shed and swallowed by the embryos (Francis, 1996; Uchida et al., 1996). The teeth probably become erect near or soon after birth, as has been found in near-term white shark embryos (Francis, 1996; Uchida et al., 1996).

Typically, embryos in a porbeagle litter are of similar size, but occasionally a large size range is encountered. Gauld (1989) found one litter with embryos ranging from 55.6 to 65.0 cm, and Shann (1923) reported a litter with a range of 38.1–50.9 cm. The runts in our two litters had small quantities of stomach and intestinal contents, and small livers, but were otherwise developing normally. This suggests that sibling competition may occur when a dominant embryo with its snout nuzzled into the anterior end of the uterus consumes most of the egg capsules as they pass into the uterus, leaving few for its sibling. However, all four embryos are usually adequately nourished, and the two embryos in each uterus are usually oriented in opposite directions. This suggests that the direction of orientation within the uterus may be a problem only if the mother is unable to produce enough egg capsules to satisfy both embryos.

At birth, embryos may still have yolk in their stomachs. Near-term white shark embryos have been reported with either empty (apart from some ingested teeth and denticles) or yolk-filled stomachs (Francis, 1996; Uchida et al., 1996). Near-term shortfin mako embryos and new-born sandtiger sharks may also have small amounts of yolk in their stomachs (Cadenat, 1956; Bass et al., 1975; Gilmore et al., 1983; Mollet et al.[11]). Along with the energy stored in the liver, this yolk supplies the nutritional needs of the embryos until they learn to feed. However, the livers of porbeagle embryos never exceeded 10% of the yolk-free embryo weight, compared with 13.5–18.6% in near-term white shark embryos (Francis, 1996; Uchida et al., 1996).

Hubbs (1923) reported a 9.1 kg (20 lb) embryo collected in late August in Maine, USA. The weight is clearly too large to be a porbeagle embryo because they are not known to exceed 5 kg (Fig. 10). Moss[3] suggests that the embryo may have been from a sandtiger shark.

The presence of an "umbilical scar" or "yolk sac scar" in postnatal oophagous sharks has puzzled many scientists who were aware that the embryos have no placental connection to their mothers (Gilmore, 1983; Stevens, 1983; Klimley, 1985; Cliff et al., 1990, 1996; Pratt and Casey, 1990; Moreno and Morón, 1992; Francis, 1996; Uchida et al., 1996). Our observations show that distension of the stomach stretches the abdominal skin and separates subdermal muscle layers as far forward as the fifth gill slits. As yolk is consumed the stomach shrinks and the muscle layers return to their original position, leaving a scar in the pectoral-gill region. The scar is sometimes faint or absent.

In all lamnid sharks, embryos are nourished by oophagy. Contrary to earlier suggestions, there is no evidence that lamnid embryos indulge in uterine cannibalism (adelphophagy), an extreme extension of oophagy that has been confirmed only in the sandtiger shark (Gilmore, 1993). All lamnids, and most oophagous sharks, produce litters larger than two (one per uterus) (Francis, 1996), providing strong circumstantial evidence that adelphophagy does not occur in those species (Gilmore, 1993). One porbeagle embryo had nonlethal abdominal lacerations, probably resulting from incidental damage inflicted by its larger sibling while searching for egg capsules, which are about the same size as the smaller embryo's abdomen. This searching behavior could provide a mechanism for the development of adelphophagy from oophagy.

Unresolved questions

Two puzzling features of the reproduction of porbeagles demand further investigation. The first concerns

the gross abdominal distension during midgestation, a phenomenon that must create problems for the mother in accommodating them. It would seem energetically and hydrodynamically more efficient for a pregnant female to match her ovulation rate to the immediate growth and energy needs of the embryo, rather than to provide an over-supply of food during a short time period. We speculate that the answer lies in the availability of food resources. Porbeagles feed mainly on small to medium pelagic fishes and cephalopods, but also eat larger demersal teleosts and elasmobranchs (Bigelow and Schroeder, 1948; Graham, 1956; Aasen, 1961; Templeman, 1963; Stevens et al., 1983; Gauld, 1989; Yatsu, 1995). Oophagy may be an adaptation that allows pregnant porbeagles (and other oophagous species) to maximize their use of food resources that are abundant only during a short season.

The lack of a six-month phase shift between the reproductive cycles of Northern and Southern Hemisphere porbeagles is surprising, and suggests that water temperature and day length have little influence on reproduction. This may be due to porbeagles having a highly developed endothermic ability (Carey et al., 1985) which buffers body temperature against seasonal fluctuations in temperature. But why should the timing of reproduction be so similar in the two hemispheres? The shortfin mako is also endothermic, although not to such a high degree as porbeagles (Carey et al., 1985), and its reproductive cycles are six months out of phase in the two hemispheres (Mollet et al.[11]). In the northwest Atlantic, porbeagle parturition coincides with the arrival of migratory stocks of Atlantic mackerel, capelin and 0+ Atlantic herring (Moss[3]). Linking parturition with the period of peak abundance of the common prey species in each hemisphere would provide new-born young with their best chance of rapid growth and survival. Unfortunately, neither of these hypotheses can be tested, because there is no information on the existence or timing of abundance cycles of porbeagle prey in the Southern Hemisphere.

This study has clarified several important aspects of the reproductive biology of porbeagles, including the length of the gestation period, mean fecundity, length at birth, and the timing of parturition. Growth rates have been estimated for embryos and juveniles and are consistent with other studies. However considerable imprecision and uncertainty remain in all of these estimates, especially the lengths of the gestation period and reproductive cycle, and therefore the annual fecundity. Such information is crucial to the determination of stock productivity in porbeagles; therefore better estimates are required before effective stock assessment and management can be achieved.

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Abstract.—The retrospective assignment of collections of larval swordfish, *Xiphias gladius*, taken from 1973 to 1980, to water types and area of the Gulf Stream front, as well as three sets of contemporary collections taken in 1984, 1988, and 1997, indicated that larvae were collected most frequently within the western Gulf Stream frontal zone. Larval swordfish accumulate by localized hydrodynamic convergence, rather than localized spawning, and thus these rare, surface-oriented larvae are found more frequently within the frontal zone. Lengths of larval swordfish taken from curatorial collections, from contemporary collections, and from published records from the Caribbean Sea, the Gulf of Mexico, and the western North Atlantic, as well as assumptions about growth rates and Gulf Stream transport, indicated that swordfish may spawn as far north as Cape Hatteras.

Distribution of larval swordfish, *Xiphias gladius*, and probable spawning off the southeastern United States

John Jeffrey Govoni
National Marine Fisheries Service, NOAA
Southeast Fisheries Science Center
Beaufort Laboratory
101 Pivers Island Road
Beaufort, North Carolina 28516-9722
E-mail address:Jeff.Govoni@noaa.gov

Bruce W. Stender

Oleg Pashuk
Marine Resources Research Institute
South Carolina Wildlife and Marine Resources Department
Charleston, South Carolina 29412

Swordfish, *Xiphias gladius*, is a cosmopolitan and highly migratory species that spawns year-round (Grall et al., 1983). Larvae of this fish do not account for high numbers of the ichthyoplankton or ichthyoneuston, and as a consequence, data that describe their spatial distribution are sparse. Grall et al. (1983) concluded from available data that, in the western North Atlantic, small larvae (<10 mm) occur most frequently in the eastern Caribbean and in the Straits of Yucatan and Florida in November, and that larger larvae (>10 mm) occur most often there, as well as in the Gulf Stream north to Cape Hatteras from January to March. Both length groups occur primarily in surface water over depths deeper than 200 m (Markle, 1974). Tibbo and Lauzier (1969) first speculated that larvae may be associated with horizontal temperature and salinity gradients. The collection of larvae along the apparent edges of the Gulf Loop Current in the Gulf of Mexico (Richards and Potthoff, 1980) and the Gulf Stream in the Atlantic (Potthoff and Kelley, 1982; Post et al., 1997) supports the notion that larvae occur in greater abundance within frontal zones. Yet, the rarity of larvae has hindered a clear understanding of such coarse and fine scale (Haury et al., 1978) spatial distribution.

The scarcity of larval swordfish has obscured an understanding of their spawning pattern as well. The mesoscale pattern of larval distribution (Grall et al., 1983) implies that swordfish spawn in the Caribbean and the Straits of Yucatan and Florida, and that their larvae are carried northward by the Gulf Stream. Occasional small larvae taken in the Atlantic imply that swordfish may spawn as far north as Cape Hatteras (Markle, 1974). Large, and presumably older larvae in any location may be the result either of local spawning and subsequent retention or of transport from a distant spawning locale. Small larvae at a specific locale must be the exclusive result of local spawning.

Hydrated oocytes within the ovaries of adult females indicate that swordfish spawn south of the Sargasso Sea, in the northern Caribbean Sea, and the Straits of Florida (Arocha and Lee, 1995), although Squires (1962) has suggested that

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spawning may occur as far north as Cape Hatteras. Planktonic eggs have not been identified in the western North Atlantic.

Effective fisheries management requires knowledge of both the spatial distribution of larvae and the distribution of spawning adults. Contemporary stock status ascertained by virtual population analysis can be calibrated with larval abundance estimates, even for species with rare larvae (e.g. McGowan and Richards, 1989; Scott et al., 1993). Because this calibration depends on accurate estimates of larval abundance, the spatial and temporal distribution of larvae must be known. Knowledge of spawning distribution would be the first step toward protection of spawning habitat and, perhaps, the restriction of fishing within spawning seasons and locales.

Here we examine the coarse- and fine-scale distribution, and the lengths of swordfish larvae off the southeastern United States. Our focus is on the influence of the Gulf Stream in shaping the spatial distribution of larvae and in the determination of probable spawning locales.

Methods

We examined published records of larval swordfish (Arata, 1954; Arnold, 1955; Tibbo and Lauzier, 1969; Markle, 1974; Post et al., 1997), data from the Marine Resources Monitoring, Assessment and Prediction Program (MARMAP), data and specimens from the Southeast Area Monitoring and Assessment Program (SEAMAP), and new data from three surveys conducted between the Florida Straits and Cape Hatteras in 1984, 1988, and 1997 (CF8406, CH8807, and CH9703).

Spatial distribution of species

For spatial distribution, we examined exclusively neuston collections (the upper 0.5 m of water), because swordfish are surface-oriented as are larvae. Although some swordfish larvae have been collected in nets that fished principally below the surface (Grall et al., 1983), most have been collected at the surface (Tåning, 1955; Yabe et al., 1959; Gorbunova, 1969; Nishikawa and Ueyanagi, 1974). All swordfish larvae collected by MARMAP, SEAMAP, and CH8807 were collected in the neuston, none in accompanying, subsurface ichthyoplankton collections. Small larvae were occasionally taken from below the surface in CH9703, but these nets fished obliquely from 20 m and larvae were likely captured when nets were near the surface.

Collections of larvae were classified to water mass (or type)—shelf water (including Georgia water, Carolina Capes water, and occasionally Virginia coastal water [Pietrafesa, 1989]) or Gulf stream water—by applying measured hydrographic characteristics to the classifications of Matthews and Pashuk (1986) for MARMAP and CF8406 collections, and Pietrafesa et al. (1985) for CH8807 and CH9703 collections. Frontal zone water is a mixture of these water masses (Hitchcock et al., 1994). South of Cape Hatteras the Gulf Stream courses north-northeastward in juxtaposition with shelf water. Classically, the definition of the Gulf Stream front is a dynamic one: the Gulf Stream front is the point where the pressure gradient between Sargasso Sea water and slope water (north of Cape Hatteras) or shelf water (south of this Cape) is zero (Stommel, 1966). Practically, observed horizontally compressed surface isotherms and isohalines, accompanied by sharp discontinuities in sea-surface texture and color, define the Gulf Stream frontal zone (Olson et al., 1994). In our study, we used this observational definition to assign the surface position and to classify the water of the Gulf Stream frontal zone. Surface Gulf Stream water at its western front between the Florida Straits and Cape Hatteras has characteristic temperatures that range from 21° to 24°C in winter and from 27° to 29°C in summer, salinities that range from 35.7 to 36.4 psu and vary little seasonally, dissolved oxygen values that range from 4.5 to 5.0 mL/L, and nitrate values of 1.0 µM/L (Atkinson, 1985; Schmitz et al., 1993; Hitchcock et al., 1994; Xie and Pietrafesa, 1995). Shelf water is cooler and less saline (Pietrafesa et al., 1985). The course of the Gulf Stream along the continental shelf break is unstable; it meanders onshore and offshore and projects intrusions, filaments, and eddies onto the shelf (Pietrafesa, 1989; Lee et al., 1991). These processes complicate the position and distort the configuration of the frontal zone.

Retrospective examination of 1163 collections taken from the Straits of Florida to Cape Hatteras and offshore from the 9 m to 2000 m isobath (Fig.1) from 1973 to 1980 (MARMAP) afforded the determination of coarse-scale distribution. These collections were taken with neuston nets of two different dimensions and meshes: a 1.0 × 0.5 m net with 505-µm mesh and a 2.0 × 1.0 m net with 947-µm mesh. Both meshes collect swordfish larvae, which are reported to be at least 4 mm at hatching (Sanzo, 1910; Yasuda et al., 1978). Neuston nets were towed for 10 min at 5.6 km/h. Collections were made in all four seasons and at all times of day and night. Larvae were preserved in 5% formalin solution. The probable location of the Gulf Stream front for these collections was determined from 1) advanced, very high resolution, infra-red radiometer (AVHRR) satellite images of sea-surface temperature (SST) taken from 1976 to 1980; 2) expendable bathythermograph (XBT) profiles taken from 1973 to 1980;

3) sea-surface salinity, dissolved oxygen, and nitrate concentrations taken along with neuston collections (Mathews and Pashuk, 1986); 4) records of the National Oceanographic Data Center; and 5) records of U.S. Coast Guard visual observations from aircraft. The width of the frontal zone, and consequently the area occupied by frontal zone water, was determined as three times the standard deviation of the mean course of the front, i.e. ±10 nautical miles (18.5 km) of the estimated location of the frontal axis (Olson et al., 1983).

Forty-seven collections taken from 22 to 24 June 1984 with a 1.0 × 0.5 m neuston net with 909-µm mesh towed for 10 min at 5.6 km/h in the Gulf Stream and within its western frontal zone between Cape Canaveral and Cape Hatteras (CF8406) afforded an examination of coarse scale differences in occurrence of swordfish larvae within the frontal zone and in the body of the Gulf Stream. Six collections were taken during night, the remainder during the day. Larvae were preserved in 5% formalin. A sea-surface temperature plot, generated from composite AVHRR images of SST compiled on 20 June 1984 (Fig. 2), in conjunction with continuous temperature and salinity values from a hull-mounted thermosalinometer, was used to determine the position of the Gulf Stream and its frontal zone and to classify these collections to water mass.

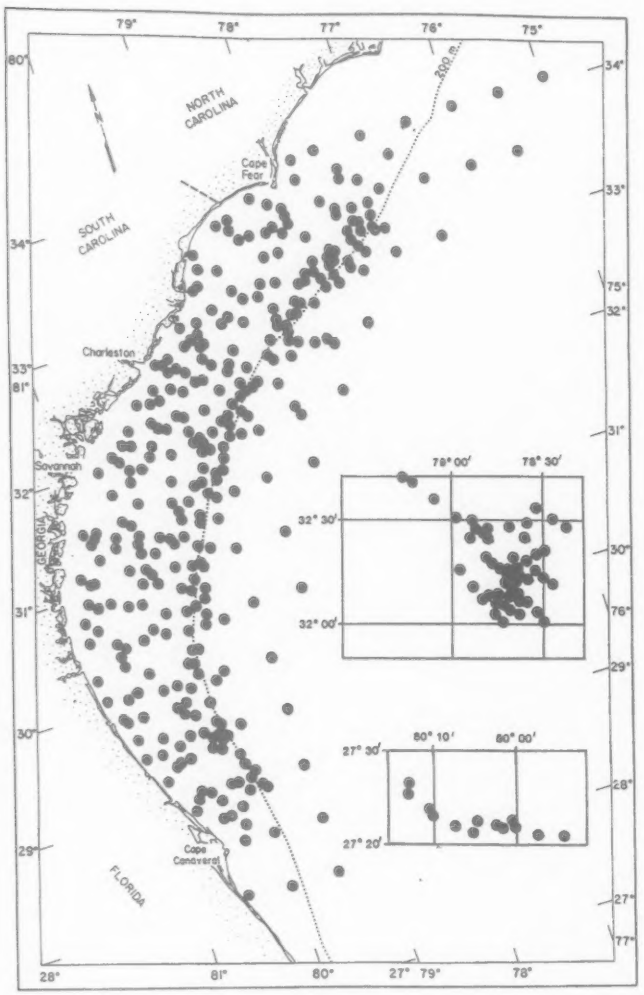

Figure 1

Nominal stations occupied by the Marine Resources Monitoring Assessment and Prediction Program from 1973 to 1980; each station was occupied at least once, some more than once.

One hundred and fifty-six collections (CH8807), taken from 13 to 24 September 1988 with a 2.0 × 1.0 m neuston net with 947-µm mesh along six cross-shelf and cross-slope transects (encompassed by 31°30.4N/080°16.5W, 30°45.0N/078°411.5W, 32 24°30.0N/076°19.5W, and 33°16.0N/077°54.0W), afforded examination of coarse- and fine-scale distribution of larval swordfish within and about the Gulf Stream frontal zone (Fig. 3). Neuston nets were towed for 10 min at 5.6 km/h. Ten stations were occupied along each transect (60 stations); 96 additional neuston collections were taken within the Gulf Stream or within its frontal zone. The 60 collections along transects were 18.5 km apart, whereas 94 of the additional 96 collections within the frontal zone or body of the Gulf Stream were clustered in groups of four to ten, with collections about 1 km apart. Surface salinity, two-dimensional sections of isotherms derived from XBT casts taken at each station along transects, and AVHRR images of SST were used to classify collections.

Six neuston collections taken on 31 May 1997 (CH9703) with a 2.0 × 1.0 m neuston net with 947-µm mesh within the Gulf Stream frontal zone in an area encompassed by 33°52.94'N/076°23.89'W, 33°52.30'N/076°24.77'W, and 33°52.92'N/076°22.27'W afforded examination of fine-scale distribution of larval swordfish within the Gulf Stream frontal zone (Fig. 4). Nets were towed for 10 min at 5.6 km/h, twice in the Gulf Stream frontal zone, and twice each on the shelf and Gulf Stream sides of the front. Larvae were preserved in 95% ethanol. Sea-surface temperature from a hull-mounted thermister in conjunction with XBT profiles and AVHRR images of SST were used to classify collections.

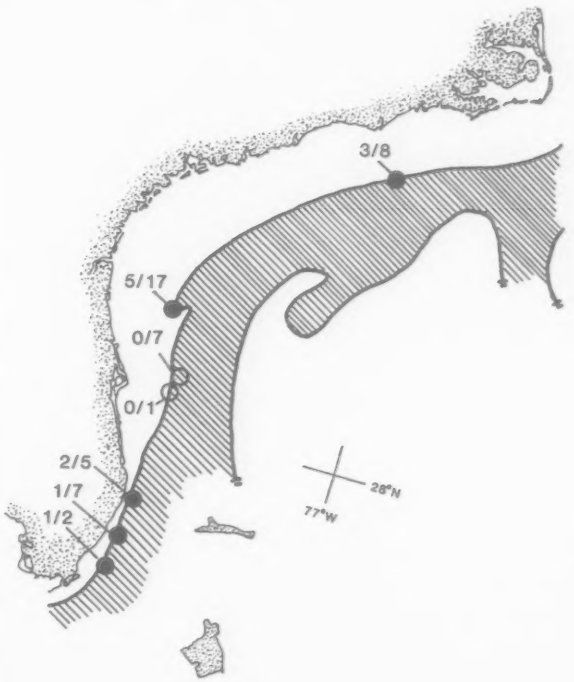

Figure 2

The position of the Gulf Stream (diagonal line pattern) illustrated from a 3-d average of sea-surface temperatures compiled on 20 June 1984 with data from infrared sensors on a Global Stationary Environmental Satellite operated by the National Weather Service (closed circles denote approximate locations of multiple neuston collections where swordfish larvae were present; open circles, locations where swordfish larvae were absent; fractions are the number of positive collections over the total number of collections).

Length of larvae

The overall distribution of the length of swordfish larvae in the Caribbean Sea, Gulf of Mexico, and off the southeastern Atlantic coast of the United States provided a view of the occurrence of the smallest larvae. Because most published have records reported either standard length (SL) or total length (TL), both measures are reported in our study. We employed no conversion, although the length of the caudal finfold or fin ranges from 3% to 12% of TL for larvae from 6 to 192 mm (calculated from Arata [1954]). We employed no correction for the shrinkage of larvae due to death or preservation, because preservation fluid was not routinely reported in the literature and some specimens were measured alive. Although the length from the anterior eye orbit to the tip of the notochord is perhaps a better measure of length in swordfish larvae, because the rostrum is often broken (Potthoff and Kelley, 1982), this measure was not uniformly available in published records. Standard or total lengths (anterior tip of the upper jaw to the posterior tip of the notochord or hypural plate (SL) or caudal fin (TL)) of swordfish larvae and locations of collection were taken from Arata (1954), Arnold (1955), Tibbo and Lauzier (1969), Markle (1974), Post et al. (1997) and MARMAP. Standard length was measured on larvae from SEAMAP, CF8408, CH8807, and CH9703 (from neuston as well as plankton collections taken within the Gulf Stream frontal zone with a MOCNESS (Wiebe, et al., 1976) system with 505 m mesh nets), and on a larva from a neuston collection taken in a *Sargassum* raft at 33°38.7'N/076°02.7'W in 1991. Specimens were considered larvae if they were <190 mm SL or TL, because

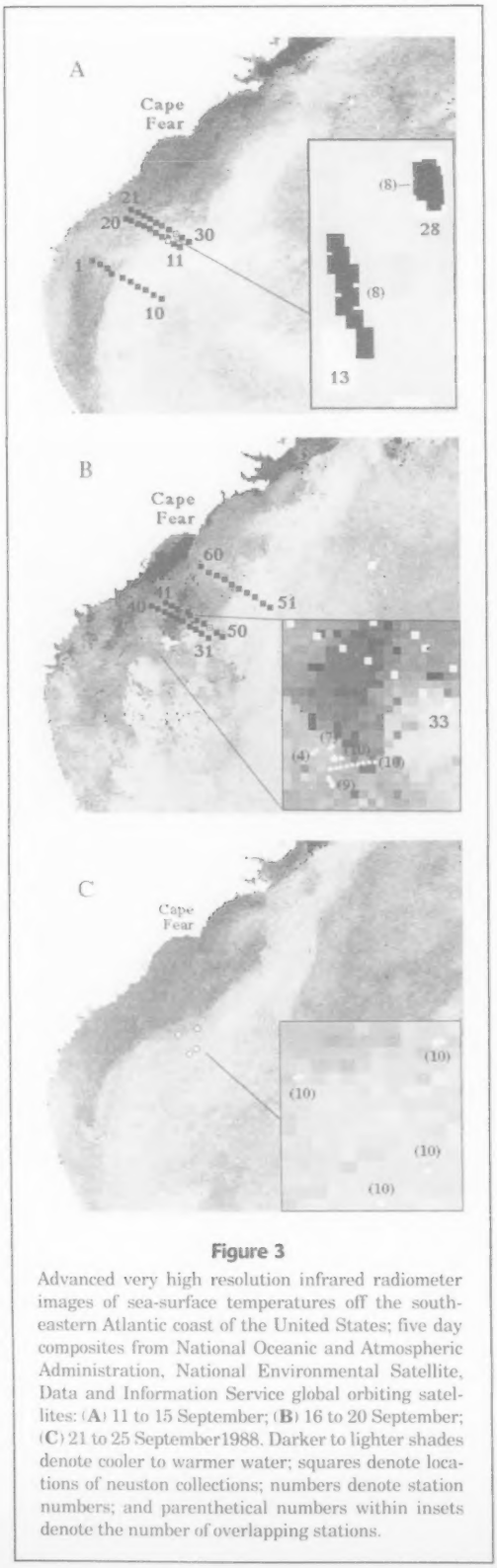

Figure 3

Advanced very high resolution infrared radiometer images of sea-surface temperatures off the southeastern Atlantic coast of the United States; five day composites from National Oceanic and Atmospheric Administration, National Environmental Satellite, Data and Information Service global orbiting satellites: (**A**) 11 to 15 September; (**B**) 16 to 20 September; (**C**) 21 to 25 September1988. Darker to lighter shades denote cooler to warmer water; squares denote locations of neuston collections; numbers denote station numbers; and parenthetical numbers within insets denote the number of overlapping stations.

swordfish retain larval characters (lower jaw at least half as long as the rostral cartilage; preorbital, supraorbital, posttemporal, and preopercular spines; enlarged and spinous dorsal and ventral scales; and a continuous and long dorsal fin) until they are at least 188 mm SL (Arata, 1954; Pothoff and Kelley, 1982; McGowan, 1988).

Approximate age of larvae

The age of swordfish larvae at length was approximated from published accounts of the age and lengths of laboratory-reared larvae, and from counts of apparent growth increments on otoliths excised from three specimens collected by CH9703. Larvae from the Mediterranean hatch at a length from 4.0 to 4.5 mm TL (measured alive) after 3 d of incubation and deplete their yolk and oil globule at a length of about 5 mm TL after 5 to 7 d at 22.5–25.2°C (Sanzo, 1910; Yasuda et al., 1978).

Results

Spatial distribution

The 1163 MARMAP collections yielded 55 swordfish larvae in 35 collections. Larvae were collected in all seasons and at all times of the day. Of the 35 collections that produced larvae, 21 were taken in the day, 5 at night, and 9 at dawn or dusk. As many as nine larvae were taken in a single collection. Between Cape Canaveral and Cape Hatteras, larvae were collected more frequently within the frontal zone of the Gulf Stream, than they were in shelf or Gulf Stream waters. Where the Gulf Stream jets through the Florida Straits (25° to 28°N latitude), larvae occurred across the narrow body of the Gulf Stream. If these Florida Straits collections are excluded from consideration, along with collections from single seasonal or annual surveys that did not produce any larvae, 21 of these 27 collections that yielded larvae were within the probable area of the Gulf Stream frontal zone, i.e. 18.5 km of the assigned position of the Gulf Stream surface frontal axis; one was in shelf water, and five were in Gulf Stream water (Fig. 5; Table 1). The probability of observing the presence or absence of larvae (calculated as frequency of occurrence), among the two water masses and the mixed frontal zone, with at least as much association was 0.000002% (Fisher's exact test, two-tailed distribution).

In June 1984 (CF8406), the frontal zone was defined by the 25° to 27°C surface isotherms. North of Florida, the western Gulf Stream front was smooth with no evidence of instabilities in the form of large intrusions (Fig. 2). Twelve of 47 collections of CF8406 yielded 16 swordfish larvae. Of these, two collections

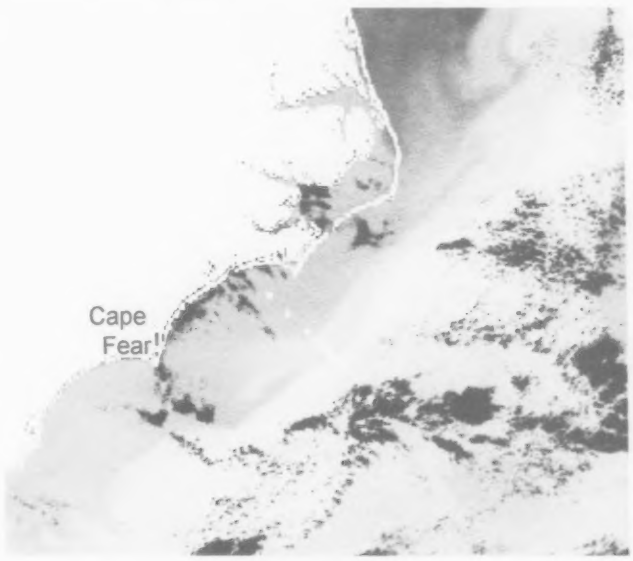

Figure 4

Advanced very high resolution infrared radiometer images of sea-surface temperatures off the southeastern Atlantic coast of the United States on 28 May 1997 from NOAA polar orbiting satellite (darker to lighter shades denote cooler to warmer water; circles denote locations of hydrographic stations occupied on 3 June 1997).

yielded more than one larva, i.e. two larvae in each. Ten collections with larvae present were within the frontal zone, whereas two collections were in the body of the Gulf Stream (Table 1). The probability of observing the presence or absence of larvae with a frequency at least this extreme was 0.176% (two-tailed test).

In September 1988 (CH8807), the Gulf Stream frontal zone was defined by 27° to 29°C surface isotherms. With the defining criterion of horizontally compressed surface isotherms within this temperature range, the width of this frontal zone ranged from 18 to 93 km. This area, however, encompassed a westward intrusion of the Gulf Stream that developed over the transect grid (Fig. 3) in the wake of the Charleston Bump, a topographic rise at the continental shelf break that typically forces an eastward deflection of the Gulf Stream (Pietrafesa et al., 1985). Isolated surface pools of 27° to 29°C water inshore of the Gulf Stream front proper (Fig. 6, B and F) indicated this intrusion. This convolution of the Gulf Stream front proper manifested three fronts across the shelf, but these were considered a composite single front for analysis. Shear zones (determined by rapid increases in the ship's set), drift lines of *Sargassum*, and discontinuities in sea-surface texture, were embedded within the frontal zone and evidenced probable convergence of surface water (Stommel, 1966; Olson et al., 1994). The 156 collections of CH8807 yielded 12 swordfish larvae. One collection yielded three larvae; another two. Larvae were collected exclusively within the frontal zone (Table 1), one at the tip of the intrusion (station 58; Fig. 6F). The probability of observing the presence or absence of larvae at least this extreme was 0.020%.

In May 1997 (CH9703), the Gulf Stream frontal zone was defined by 27° to 29°C surface isotherms. The frontal zone was moving rapidly eastward. On 23 May, 8 d before the collection of swordfish larvae within the frontal zone, an elongate filament of a Gulf Stream intrusion lay inshore of the collection area (Fig. 4). The isolated pool of 25°C water at the surface and eastward of the frontal zone (Fig. 7) indicated that this intrusion was extant on 31 May 1997 when collections of CH9703 were taken. The six neuston collections of CH9703 yielded nine larvae. One collection taken along the frontal axis and one collection taken on the Gulf Stream side of the axis yielded two larvae each; the other collection taken along the axis yielded three. Larvae were present in two collections along the frontal axis, two collections 1 km to the Gulf Stream side of the axis, and one of two collections taken 0.5 km

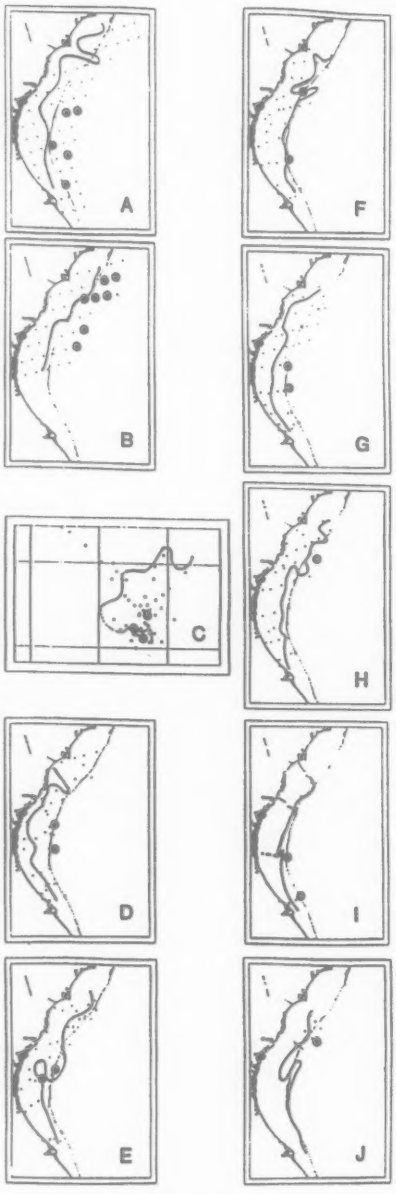

Figure 5

Position of the Gulf Stream front and locations of neuston collections of surveys when swordfish larvae were collected from 1973 to 1980 (solid lines denote assigned axis of the Gulf Stream front, small circles locations of neuston collections; large circles locations of swordfish larvae: (**A**) March 1973; (**B**) May 1973; (**C**) July 73; (**D**) November 1973; (**E**) August and September 1974; (**F**) August and September 1975; (**G**) January and February 1976; (**H**) August and September 1976; (**I**) September 1978; (**J**) April 1979.

Table 1

Contingency tables of the absence and presence of larval swordfish, *Xiphias gladius*, in Gulf Stream frontal zone, the Gulf Stream, and shelf water in the southeastern Atlantic bight off the United States.

Frequency	Frontal zone water	Gulf Stream water	Shelf water	Total
1973–80				
Absent	158	25	175	358
Present	21	5	1	27
Total	179	30	176	385
1984				
Absent	21	14	35	
Present	10	2	12	
Total	31	16	47	
1988				
Absent	77	46	24	147
Present	9	0	0	9
Total	86	46	24	156
1997				
Absent	0[1]	0[2]	1[3]	1
Present	2[1]	2[2]	1[3]	5
Total	2[1]	2[2]	2[3]	6

[1] Taken along the frontal axis.
[2] Taken along offshore side of the axis.
[3] Taken on inshore side of the axis.

to the shoreward side of the frontal axis. Larvae were evenly distributed within the frontal zone (Table 1).

Length of larvae

Swordfish larvae ranged from 2.8 mm SL to 110 mm TL. The two largest larvae, >100 mm TL, were collected in the Atlantic and in the Caribbean. The smallest and youngest larvae, those ≤5 mm SL (Fig. 8), were collected in the eastern Gulf of Mexico in the vicinity of the Gulf Loop Current between 24° and 28°N (here, 39 of 152 larvae were ≤5 mm), and off the southeast coast from Georgia north to Cape Hatteras between 30° and 35°N latitude (here, 15 of 62 larvae were ≤5 mm SL). Larval lengths were not correlated with latitude (Pearson product-moment correlation coefficient=–0.1360).

Approximate age of larvae

The smallest larva measured (after preservation in 95% ethanol), taken in the Gulf of Mexico from SEAMAP, was appreciably smaller than the length at hatching for larvae (measured alive, 27 h after fertilization) from the Mediterranean (Yasuda et al., 1978). One larva 2.8 mm SL from the eastern Gulf of Mexico, and

one 3.8 mm SL (4.3 mm TL) taken off North Carolina (CH9703), had recognizable yolk and oil globule remnants. These lengths were smaller than the reported length of larvae at the completion of yolk and oil globule absorption, about 5 mm TL for larvae (measured alive, 65 h after fertilization) from the Mediterranean (Yasuda et al., 1978).

Counts of increments on sagittae from larvae 4, 5, and 6 mm SL, taken in CH9703, were 4, 3, and 6 (increments on swordfish otoliths have not been validated as daily intervals [Price et al., 1991]).

Discussion

North of the Florida Straits, larval swordfish were collected most frequently within the western frontal zone of the Gulf Stream. This observation corroborates the speculation that larvae are associated with water within sharp horizontal gradients of temperature and salinity (Tibbo and Lauzier, 1969). Convergence of surface water is a possible mechanism for their accumulation within the Gulf Stream front. The Gulf Stream front south of Cape Hatteras is cyclonically sheared with shelf water that directly opposes Gulf Stream water (Pietrafesa et al., 1985). The retrograde, hydrographic discontinuity between Gulf Stream and shelf water and their hydrodynamic opposition results in convergence of surface water within the frontal zone (Garvine, 1980; Olson et al., 1994). Convergence of surface water has been implicated in the accumulation of adult fishes with depth-keeping abilities (Olson and Backus, 1985). Positively buoyant or surface-seeking larval fishes will be entrained in converging water and will be advected toward the front where they will accumulate as they resist downwelling along the frontal axis (Govoni and Grimes, 1992). Swordfish larvae are unquestionably surface-seeking larvae. Convergence of surface water within oceanic frontal zones should produce accumulations of larvae on spatial scales of 10 to 100 km (Olson et al., 1994). At a coarse scale, larvae were more abundant within the frontal zone; no fine-scale pattern was evident within the frontal zone. Adaptive sampling (Lo et al., 1997) may be a more efficient means of estimating larval swordfish abundance than simple random sampling, because of the rarity and the spatial aggregation of larvae.

Small swordfish larvae were collected most often in the eastern Gulf of Mexico and off the east coast of the United States as far north as Cape Lookout, North Carolina. Swordfish apparently spawn in the eastern Gulf, but the present observations corroborate the speculation of spawning off the Carolinas (Squires, 1962; Markle, 1974) as well. Off the Carolinas, larvae

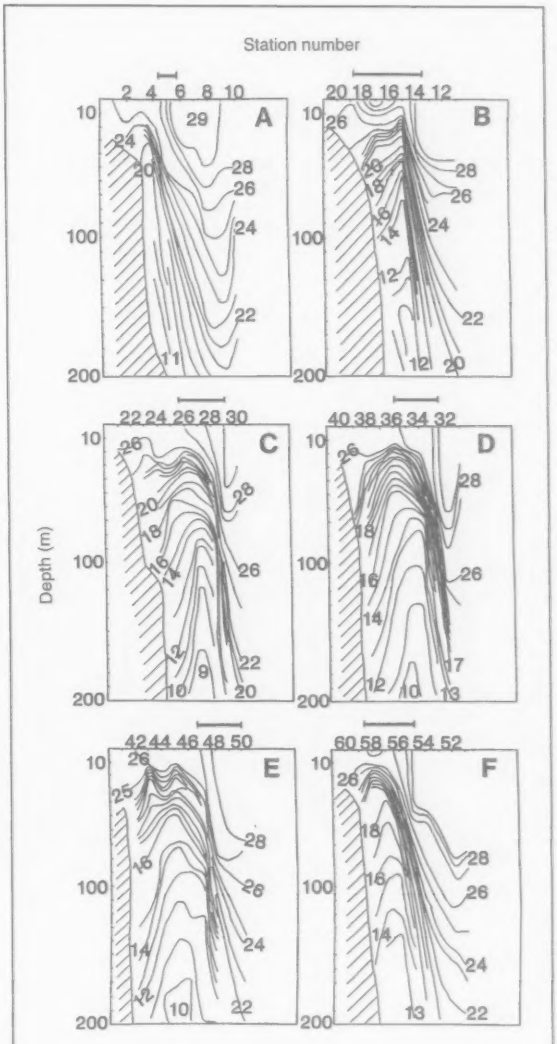

Figure 6

Sections of isotherms along transects occupied in September 1988: (**A**) 13 September; (**B**) 14 September; (**C**) 15 September; (**D**) 16 September; (**E**) 17 September; (**F**) 18 September (bars above station numbers indicate the Gulf Stream frontal zone).

5 mm SL or less occurred in 25° and 26°C water. Larvae that were 4 to 5 mm SL had 3 and 4 apparent growth increments on their sagittae. In water from 22° to 25°C, larvae that were 5 mm TL would be approximately 6 d old (Yasuda et al., 1978). Given an egg incubation period of 3 d at 24°C (Yasuda et al., 1978) and an additional 3 or 4 d for posthatch growth, along with a average axial velocity of the Gulf Stream of 1.5

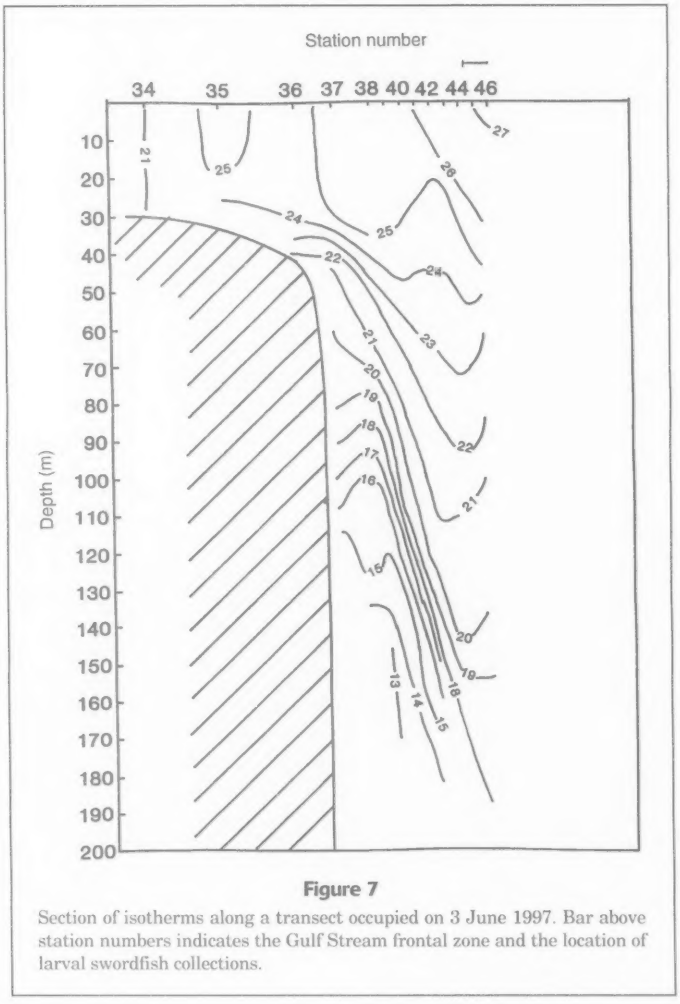

Figure 7

Section of isotherms along a transect occupied on 3 June 1997. Bar above station numbers indicates the Gulf Stream frontal zone and the location of larval swordfish collections.

m/s (Olson et al., 1994), larvae that were 4 to 5 mm SL in the Atlantic could have been transported from as far away as 900 km. A similar trajectory was projected for small larvae of bluefin tuna, *Thunnus thynnus* (McGowan and Richards, 1989). Larvae that were ≤5 mm in length, collected off North Carolina could have been spawned in the Florida Straits if they remained in the core of the Gulf Stream. Current velocities within the western Gulf Stream frontal zone, where larvae most frequently reside, are less than axial velocities (Lillibridge et al., 1990; Song et al., 1995; Limouzy-Paris et al., 1997). Further, departures from a smooth, along slope, Gulf Stream trajectory, in the form of meanders, intrusions, and filaments along the western Gulf Stream frontal zone are frequent (Pietrafesa, 1989). We collected swordfish larvae frequently within these Gulf Stream anomalies (Figs. 4–7). Water within these features veers and reverses direction (Lee et al., 1991), the result being that the northward translocation of swordfish larvae within the frontal zone is checked and their northward transport delayed. The possibility of spawning between Cape Canaveral and Cape Hatteras is real, but not certain.

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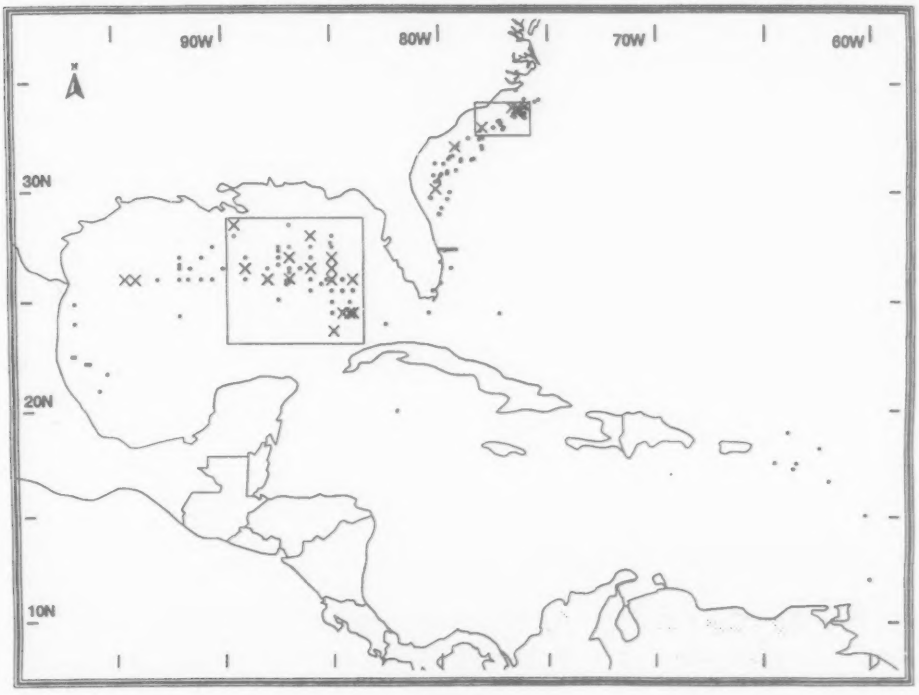

Figure 8

Locations of larval swordfish collection in the Caribbean Sea, Gulf of Mexico, and off the southeast coast of the United States north to Cape Hatteras (crosses depict larvae 2.8 through 5 mm standard (SL) or total length (TL); circles, larvae 5.1 through 110 mm SL or TL; within the box in the Gulf of Mexico, coincidental crosses represent 39 larvae ≤5 mm and 113 >5 mm SL; within the Atlantic, coincidental crosses represent 15 larvae ≤5 mm and coincidental circles 47 >5mm SL).

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A biological reference point based on the Leslie matrix

Daniel B. Hayes
Department of Fisheries and Wildlife
13 Natural Resources Building
Michigan State University
East Lansing, Michigan 48824-1222
E-mail address: hayesdan@msu.edu

Abstract.—For fish populations with an annual breeding cycle, a biological reference point based on the Leslie matrix is presented and compared with percent maximum spawning potential (%MSP) and F_{med} reference points. For deterministic population projections, the reference point is defined as the level of fishing mortality (F_{st}) that results in a Leslie matrix with a dominant eigenvalue (i.e. finite rate of increase or λ) of 1.0. It is shown that for the same input data, F_{st} is similar to a reference point based on a %MSP approach. For populations that are growing or declining, however, populations with the same λ but with different age-specific selectivities have different levels of %MSP. Previous applications of this reference point are extended to include situations where recruitment is a stochastic process. In stochastic projections, F_{st} is defined as the level of fishing mortality that results in an average finite rate of increase of 1.0. In an example with Georges Bank haddock, a deterministic analysis with mean birth and death rates resulted in an estimate of F_{st} of 0.52. The same estimate of F_{st} was obtained in a stochastic projection in which the growth rate of the mean population size was used. Stochastic projections using the mean of the finite rates of increase resulted in a lower estimate of F_{st} (0.45). When the value of recruits per unit of spawning stock biomass used in the %MSP analysis was calculated as Σrecruits/Σspawning stock biomass, the estimated reference point was the same as the stochastic projection. On the basis of these results, I recommend calculating the reference point based on a stochastic projection for which the mean of the simulated growth rates is used. A reference point based on a %MSP approach using the Σrecruits/Σspawning stock biomass results in an equivalent estimate of the reference point but does not convey important information on the expected population growth rate at higher or lower rates of fishing mortality.

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Advice to fishery managers on desirable harvest or exploitation rates is ideally based on full knowledge of fishery dynamics, including information on the fish population's stock-recruitment relationship, growth and maturation schedule, and bioeconomic considerations. With a lack of such complete information, guidance to fishery managers often takes the form of providing an estimate of fishing mortality and a comparison of that rate to one or more biological reference points (e.g. Clark, 1991; Anonymous[1]). Numerous biological reference points exist, each concerned with a somewhat different aspect of population response to harvesting. One class of reference points focuses on yield per recruit as a function of fishing mortality. The general goal of this class of reference points is to optimize harvest rates in relation to natural mortality and growth (i.e. prevent growth overfishing; Beverton and Holt, 1957). For example, F_{max} is the fishing mortality rate at which yield per recruit is maximized (Beverton and Holt, 1957). A related reference point is $F_{0.1}$, which is the fishing mortality rate where the slope of the yield per recruit curve is 10% of the slope at the origin (Gulland and Boerema, 1973). Fishing at F_{max} or $F_{0.1}$ results in maximal or nearly maximal yield from a fishery when recruitment is independent of stock size. A limitation of this class of reference points, however, is that reductions in recruitment are often evident when stocks are depleted to low levels (e.g. Overholtz et al., 1986). Thus, management advice based on F_{max} or $F_{0.1}$ can result in declines in abundance through recruitment overfishing (Sissenwine and Shepherd, 1987), ultimately resulting in reduced total yield from a stock.

As a counterpart to reference points based on yield per recruit, several reference points based on stock-recruitment considerations have been developed. The goal of these reference points is to provide a measure of fishing mortality that will likely avoid recruitment overfishing. An example of this type of reference point is F_{med} which is based on the median of the observed levels of recruits produced per unit of spawning stock biomass (R/SSB) (Sissenwine and Shepherd, 1987). The rationale behind this reference point is that fish abundance is maintained when the spawning stock biomass produced by a cohort over its lifetime is equal to the spawning stock biomass of the parent population when the cohort was spawned. Related to F_{med} is a set of reference points based on the spawning stock biomass per recruit (SSB/R) in relation to the SSB/R that would be produced if the

[1] Anonymous. 1996. Report of the 20th Northeast Regional Stock Assessment Workshop (20th SAW): SAW Public Review Workshop. Northeast Fisheries Science Center Reference Document 95-19. National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Woods Hole, MA, 52 p.

stock were not fished (Gabriel et al., 1989; Clark, 1991). These reference points are termed percent maximum spawning potential (%MSP) or spawning per recruit (SPR) reference points. As an example, Gabriel et al. (1989) found that fishing at F_{med} for Georges Bank haddock results in a SSB/R ratio of about 30% ($F_{30\%}$) of the SSB/R that would be produced if the stock were not fished.

There are several limitations to this group of reference points. First, the population-level effect of fishing above or below a given reference point is not immediately obvious. For example, if fishing at $F_{30\%}$ results in a stable population, the rate of population decline when fishing mortality results in a 20% MSP is not clear. Secondly, although the use of the median SSB/R is an attempt to determine the SSB/R ratio with a robust estimator, the effects of variability in recruitment have not been closely examined in the estimation process. There is also an implication that two fishing mortality patterns (i.e. combination of fishing intensity and age at entry into the fishery) that produce the same SSB/R will result in equivalent impacts on the population. To my knowledge, this implication has not been investigated. Finally, these reference points treat the R/SSB ratio as being independent of stock size. Although the stock-recruitment relationship for many stocks is so weak and highly variable that this is a reasonable approach (Clark, 1991), this assumption should be examined on a case-by-case basis.

The goal of this paper is to explore a method for computing a reference point based on stock-recruitment data that overcomes some of the limitations of F_{med} and related reference points concerned with recruitment overfishing. In particular, this method allows for 1) a direct determination of the population-level impact of fishing above or below the reference point and, 2) incorporation of information on recruitment variability into the estimated reference point. This method does not, however, take into account any curvature in the stock-recruitment relationship (i.e. this method assumes that recruitment is proportional to spawning stock biomass). Although this assumption is not always met, the reduction in abundance that occurs for most exploited fish stocks results in a reduced magnitude of density-dependent effects on recruitment. Thus, the use of this reference point is likely to be reasonable for fish stocks that have already been exploited (Francis, 1997).

Methods

The proposed method is founded on an eigenvalue analysis of Leslie matrices representing the population's dynamics under exploitation. As such, the model is specifically intended for use for fish with a single breeding season per year. The underlying model is based on one developed by Quinn and Szarzi (1993), which led to determination of the fishing mortality (F_{st}) that resulted in a stationary population in a Leslie matrix setting. Their results are extended by examining the effects of recruitment variability on the reference point and the relationship between this method and %MSP methods. This model is conceptually similar to those used for environmental impact assessment of power plants (e.g. DeAngelis et al., 1977; Cohen et al., 1983; Goodyear and Christensen, 1984) but differs in that it focuses on sustainable harvest rates across several age classes, whereas environmental impact assessment models typically focus on mortality of early life stages. Related methods have also been presented by Getz and Haight (1989). Their methods, however, are not explicitly framed toward providing a reference point for a fishery. Further, their methods are based on catch (in numbers or weight) whereas my methods are based on fishing mortality rates.

Developing a Leslie matrix representation of harvesting: deterministic case

Consider initially a population with no harvest, and where abundance estimates are available on an annual basis at the time of breeding. If we assume that the vital rates (i.e. age-specific reproduction and survival) are constant, the dynamics of the population can be represented by a Leslie matrix L_u; (see Table 1 for a list of symbols and their definitions):

$$L_u = \begin{bmatrix} E(0) & E(1) & E(2) & \dots & E(s) \\ S(0) & 0 & 0 & \dots & 0 \\ 0 & S(1) & 0 & \dots & 0 \\ 0 & 0 & S(2) & \dots & 0 \\ \dots & \dots & \dots & \dots & \dots \\ 0 & 0 & 0 & S(s-1) & 0 \end{bmatrix}$$

where $E(i)$ is age-specific fecundity, $S(i)$ is the annual survival rate from age i to $i+1$ and s is the maximum age. With this projection matrix, the population at time $t+1$ can be determine from the population at time t by the equation:

$$N_{t+1} = L_u N_t.$$

Note that estimates of natural mortality rate are also necessary to compute the previously mentioned reference points and are typically available for species where quantitative stock assessments are performed. Also note that although age-specific fecundity is not always measured in fish stock assessments, spawning stock biomass is commonly used as a surrogate

Table 1
Symbols used and their definitions.

	Definition
$E(i)$	Fecundity at age i.
$F(i)$	Instantaneous fishing mortality rate from age i to $i+1$.
F	Fishing mortality rate on fully recruited age class(es).
F_{st}	Sustainable fishing mortality rate on fully recruited age class(es).
L_e	Leslie matrix representing exploited population.
L_u	Leslie matrix representing unexploited population.
λ_e	Dominant eigenvalue of L_e.
λ_u	Dominant eigenvalue of L_u.
$M(i)$	Instantaneous natural mortality rate from age i to $i+1$.
$N(i)$	Number at age i.
N_t	Total population size at time t.
i	Age index.
$PM(i)$	Proportion of females mature at age i.
R/SSB	Recruits per unit of spawning stock biomass.
SSB	Spawning stock biomass.
SSB/R	Spawning stock biomass per recruit
s	Maximum age.
$S(i)$	Annual survival rate from age i to $i+1$.
t_c	Age at recruitment when vulnerability to the fishery is knife-edge.
$W(i)$	Weight at age i.
$X(i)$	Fecundity equivalents for fish of age i.

(see Rothschild and Fogarty (1989) for cautions on this practice, however). Because data on spawning stock biomass are more commonly presented in fishery assessments than data on fecundity, I will present the model using age-specific fecundity equivalents (i.e. spawning biomass of individual fish) rather than fecundity. In this representation, the survival rate of age-0 fish is expressed as recruits per unit of spawning stock biomass (R/SSB) rather than as actual survival rate from egg to age 1, and SSB is used in place of egg production. It is easily shown that the use of R/SSB and SSB in the Leslie matrix is algebraically equivalent to using fecundity and survival from egg to age 1.

Given the mapping of the vital rates $[E(i), S(i)]$ of the unexploited population into a Leslie matrix, it is straightforward to represent the dynamics of the population under exploitation. Observe that for the unexploited population $S(i)=e^{-M(i)}$, where $M(i)$ is the instantaneous natural mortality rate. For an exploited population, $S(i)=e^{-(M(i)+F(i))}$ where $F(i)$ is the age-specific instantaneous rate of fishing mortality. The Leslie matrix for the population under exploitation (L_e) is thus formed in exactly the same manner as for the unexploited population except that annual survival rates are decreased through fishing mortality.

It is important to emphasize that in this analysis, fecundity and natural mortality (including age-0 survival or R/SSB) are assumed to be constant. As such, the determination of a reference point based on an analysis of L_e is valid over the range of stock sizes for which these age-specific fecundity and mortality rates apply.

Methods of analyzing the Leslie matrix are well established (e.g. Keyfitz, 1977; Caswell, 1989). Properties of the Leslie matrix under certain regularity conditions include the following:

1 The Leslie matrix has at least one positive root (eigenvalue);
2 The largest of these roots (the dominant eigenvalue or λ) determines the population growth rate, except in cases where the population is inherently cyclical and the largest roots are of equal magnitude;
3 If $\lambda > 1$ the population will increase;
If $\lambda = 1$ the population will remain steady;
If $\lambda < 1$ the population will decrease.

(Pielou, 1974; Caswell, 1989; Getz and Haight, 1989). Of particular importance to this reference point is the dominant eigenvalue which, in the deterministic case, is sufficient to determine the long-term trend in population abundance (Keyfitz, 1977; Cohen et al., 1983). Given these properties, the following assertion for the deterministic case can be made:

1 A population under exploitation can maintain itself at or above a given level of abundance only if the dominant eigenvalue of L_e (i.e. λ_e) ≥ 1.

From this assertion arises the proposed reference point: F_{st} (for F steady, after Quinn and Szarzi, 1993) is a fishing mortality pattern where $\lambda_e = 1$. Note that F_{st} is actually a vector comprising two components: an overall level of fishing mortality (often termed fully recruited F) and the relative fishing mortality between age classes (often referred to as the partial recruitment vector or selection pattern), and that F_i = (fully recruited F) × (selection on age i). By convention, I will use the fully recruited F as an index of the overall level of fishing mortality, but stress that specification of the partial recruitment function is also necessary to determine the impact of harvesting on a population. Also note that there is an infinite set of fishing mortality patterns for which the condition that $\lambda_e=1$ is satisfied. For a given partial recruitment func-

tion, however, only one level of fishing will satisfy this condition. Note that the converse is not true; for a given level of fully recruited fishing mortality, numerous partial recruitment functions can satisfy the above condition. Because of the nature of these relationships, I will focus on those situations where the partial recruitment function is specified and solve for the level of fishing that is sustainable. Once the selection pattern and fully recruited fishing mortality are set, λ_e can be found by the power method as described by Johnson and Riess (1981).

Example of maintenance fishing mortality: deterministic case

Data from Georges Bank haddock (*Melanogrammus aeglefinus*) are used to illustrate the computation and application of this reference point. For ease of discussion, I first present general results assuming knife-edge recruitment to the fishery at age t_c with full vulnerability thereafter.

Age-specific fecundity equivalents ($X(i)$; Table 2) were computed as

$$X(i) = W(i) \times PM(i),$$

where $W(i)$ = mean weight (kg) at age i; and
$PM(i)$ = proportion of females mature at age i.

and spawning stock biomass was computed as the product of fecundity equivalents and number of fish at age. Mean weight at age ($W(i)$) and proportion of females mature ($PM(i)$) reported by O'Brien and Brown[2] were used in this analysis. The instantaneous natural mortality rate ($M(i)$) of haddock age 1 and older is 0.2 (Clark et al., 1982), and a maximum age of 15 was used following Gabriel et al. (1989).

I computed annual R/SSB (Table 3) from the ratio of number of female fish at age 1 to their parental female spawning stock biomass (Clark et al., 1982; O'Brien and Brown[2]; Hayes and Buxton[3]) for the period 1931–94. For the entire data series, R/SSB averaged 0.5902. As noted by Gabriel et al. (1989), however, the R/SSB ratio (reflecting age-0 survival) appears to have declined following the collapse of the Georges Bank haddock stock during the early 1960s.

Table 2

Age-specific characteristics of Georges Bank haddock. Fecundity equivalents (i.e. spawning biomass per individual) are denoted as X, instantaneous natural mortality rate as M, and partial recruitment as PR. The proportion mature and mean weights at age are from O'Brien and Brown (see Footnote 2 in the main text).

Age	Proportion mature	Mean weight (kg)	X	M	PR
0	0.00	—	0.000	—	0.00
1	0.08	0.486	0.039	0.2	0.00
2	0.46	0.676	0.311	0.2	0.07
3	0.88	1.197	1.053	0.2	0.65
4	1.00	1.621	1.621	0.2	1.00
5	1.00	2.021	2.021	0.2	1.00
6	1.00	2.424	2.424	0.2	1.00
7	1.00	2.780	2.780	0.2	1.00
8	1.00	3.192[1]	3.192	0.2	1.00
9	1.00	3.548[1]	3.548	0.2	1.00
10	1.00	3.924[1]	3.924	0.2	1.00
11	1.00	4.297[1]	4.297	0.2	1.00
12	1.00	4.667[1]	4.667	0.2	1.00
13	1.00	5.034[1]	5.034	0.2	1.00
14	1.00	5.398[1]	5.398	0.2	1.00
15	1.00	5.758[1]	5.758	0.2	1.00

[1] Predicted from von Bertalanffy growth equation fitted to observed data for ages 1 to 7.

At present the causes of the decline in the R/SSB ratio are not known. Several hypotheses explaining the observed decline in R/SSB have been put forth, including depensatory mortality on age-0 haddock (Collie and Spencer, 1993), changes in oceanographic conditions (Myers and Pepin, 1994), and increased predation or competition with elasmobranchs (Collie and Spencer, 1993). Although the cause is not known, a crucial consideration is the choice of an appropriate time period where R/SSB is representative of current population abundance and biomass and current environmental conditions.

One strategy to obtain a mean R/SSB value representative of "current" conditions is to average R/SSB from the most recent data point back several years. The philosophy behind this strategy is to smooth annual variations in R/SSB by averaging over a sufficiently long time period. The problem, however, is to define a time period sufficiently long to achieve adequate precision without introducing excessive bias. Averages over short time periods suffer from low precision and can vary considerably because of annual variation in R/SSB. If averages are taken over a time period spanning a wide range of population levels or

[2] O'Brien, L., and R. W. Brown. 1996. Assessment of the Georges Bank haddock stock for 1994. Northeast Fisheries Science Center Reference Document 95-13. National Marine Fisheries Service, Northeast Fisheries Science Center, Woods Hole, MA. 107 p.

[3] Hayes, D. B., and N. G. Buxton. 1991. Assessment of the Georges Bank haddock stock. Papers of the Northeast Regional Stock Assessment Workshop, Research Document SAW13/1, 126 p.

Table 3

Stock-recruitment data for Georges Bank haddock, 1931 to 1990. Data for 1931 to 1962 Hayes and Buxton (see Footnote 3 in the main text), and from 1963 to 1994 from O'Brien and Brown (see Footnote 2 in the main text).

Year	Spawning stock biomass (t) females only	Number of age-1 females produced (millions)	R/SSB	Year	Spawning stock biomass (t) females only	Number of age-1 females produced (millions)	R/SSB
1931	75,767	22.793	0.3008	1963	82,128	235.939	2.8728
1932	61,586	26.052	0.4230	1964	64,278	16.577	0.2579
1933	52,643	30.239	0.5744	1965	72,512	2.013	0.0278
1934	47,187	29.436	0.6238	1966	90,260	6.426	0.0712
1935	52,104	29.323	0.5628	1967	56,051	0.211	0.0038
1936	54,967	53.173	0.9674	1968	37,546	0.494	0.0132
1937	52,872	39.626	0.7495	1969	25,589	2.330	0.0911
1938	60,434	29.875	0.4943	1970	19,255	0.184	
1939	70,067	55.903	0.7979	1972	13,456	9.707	0.7214
1940	67,418	57.052	0.8462	1973	6,135	5.270	0.8590
1941	76,187	30.731	0.4034	1974	10,906	3.827	0.3509
1942	77,248	11.783	0.1525	1975	9,190	51.616	5.6165
1943	75,511	32.411	0.4292	1976	11,031	6.891	0.6247
1944	68,957	20.806	0.3017	1977	20,717	3.029	0.1462
1945	62,785	46.548	0.7414	1978	34,427	41.941	1.2183
1946	65,712	30.486	0.4639	1979	33,841	5.052	0.1493
1947	57,269	16.676	0.2912	1980	31,703	3.600	0.1136
1948	55,659	62.918	1.1304	1981	27,757	1.230	0.0443
1949	45,791	29.447	0.6431	1982	22,682	1.519	0.0670
1950	55,919	52.810	0.9444	1983	17,531	8.548	0.4876
1951	54,039	21.795	0.4033	1984	12,583	0.865	0.0687
1952	55,497	66.748	1.2027	1985	10,313	7.233	0.7013
1953	54,772	26.276	0.4797	1986	10,186	0.882	0.0866
1954	65,764	46.429	0.7060	1987	9,540	8.024	0.8411
1955	68,949	30.534	0.4428	1988	8,781	0.580	0.0661
1956	78,979	31.155	0.3945	1989	9,061	1.244	0.1373
1957	75,364	29.630	0.3932	1990	9,762	0.991	0.1015
1958	75,995	63.270	0.8326	1991	8,682	4.125	0.4751
1959	77,394	61.685	0.7970	1992	6,231	7.219	1.1586
1960	93,667	26.950	0.2877	1993	5,001	3.754	0.7507
1961	113,366	19.368	0.1708	1994	7,324	3.938	0.5377
1962	109,001	95.348	0.8747				

spanning a trend in environmental conditions, the estimate of future R/SSB may be biased. For this stock, mean R/SSB for periods of 5 to 15 years appear relatively stable. Over longer periods of time, mean R/SSB shows an upward trend, punctuated by sharp increases corresponding to the 1963 and 1975 year classes when R/SSB ratios were much higher than in any other years. Because of the trends observed over longer periods of time, I chose the time period from 1976 to 1994 as representative of "current" conditions for R/SSB, or equivalently, age-0 survival. For this time period, R/SSB averaged 0.4092, and had a median of 0.1493.

Based on the mean R/SSB for 1976 to 1994, the haddock population would be expected to grow at a rate of 18.0% per year with no exploitation (i.e. λ_u=1.180). For knife-edge recruitment, I computed λ_e for fishing mortality rates ranging from 0 to 2.0 (Fig. 1) and for ages at entry (t_c) from 1 to 5 years and for the commercial fishery age selectivity observed in 1993–94 (Table 2). Additionally, I determined F_{st} and %MSP (following the methods of Gabriel et al., 1989) for each age at entry (Table 4). It is apparent from this analysis that as age at entry is delayed, the impact of fishing on the population is decreased (Fig. 1). Thus, higher fishing mortality rates can be sustained when recruitment to the fishery is delayed (Table 4). In fact, when the age at entry is 5 or greater, any level of fishing mortality is sustainable.

These conclusions are not new; analysis of SSB/R yields similar insights into the response of populations

to harvesting. The analysis of the Leslie matrix offers information not available in the analysis of SSB/R, however. First, the consequences of overfishing or "underfishing" are clearly evident from the graph of λ_e against fishing mortality rate. For example, for an age at entry of 3 years, F_{st} is 0.465. If the fishing mortality rate was limited to 0.20 (for example), the population would be expected to increase at a rate of about 8.8% per year (Fig. 1). Likewise if fishing mortality was increased to 1.0, the population would be expected to decline at a rate of about 10.3% per year (Fig. 1).

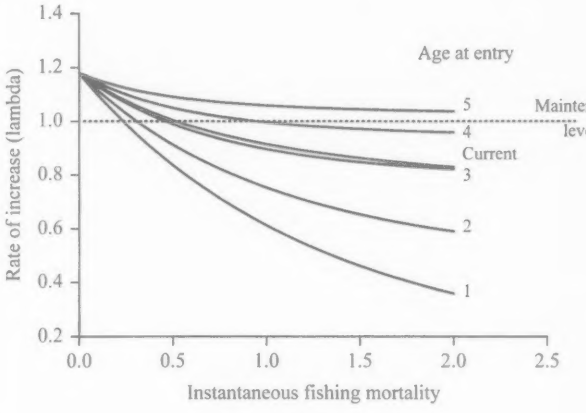

Figure 1

Rate of population increase (lambda) in relation to instantaneous fishing mortality (F) for a range of age at entry into the fishery.

When %MSP is plotted against lambda resulting from various levels of fishing mortality and ages at entry into the fishery, it is apparent that equal %MSP values are obtained for the same lambda only at two points along each of the curves. The first point is for the unfished population when lambda is at a maximum and there is 100% MSP. The second point where all %MSP values are equal is when lambda is equal to 1.00 (Fig. 2). These results demonstrate the assertion that fishing mortality rates that result in equal %MSP values do not necessarily result in the same population dynamics (i.e. the same rate of increase or decrease). The reason for this disparity is that in a growing or declining population, the timing of reproduction during the lifetime is important, as well as the total lifetime egg production. For example, when a population is growing, earlier realization of lifetime spawning potential contributes more to population growth than later reproduction. This relationship is evident when the formula for lifetime spawning stock biomass (on which %MSP is based) is compared to the formula for reproductive value, upon which the rate of population change depends. Observe that lifetime spawning stock biomass per newborn individual is (Gabriel et al., 1989)

$$SSB/N_0 = S(0)W(1)PM(1) + S(0)S(1)W(2)PM(2) + S(0)S(1)S(2)W(3)PM(3) + \ldots .$$

This formula is equivalent to that for the "net reproductive rate" (Caswell, 1989) which is the expected number of offspring produced by a newborn over its lifetime. With the above notation, the reproductive value (Caswell, 1989) of an age-1 individual can be expressed as

$$\textit{Reproductive value} = S(0)S(1)W(2)PM(2)\lambda^{-1} + S(0)S(1)S(2)W(3)PM(3)\lambda^{-2} + S(0)S(1)S(2)S(3)W(4)PM(4)\lambda^{-3} + \ldots .$$

Table 4

Sustainable fishing mortality (F_{st}) and %MSP as a function of age at entry (t_c) for Georges Bank haddock.

t_c	F_{st}	%MSP
1	0.236	27.20
2	0.309	27.20
3	0.465	27.20
Current	0.519	27.20
4	0.956	27.20
5	>2.0	—

These formulae are similar except for the addition of the term λ^{-i}, where i is the age index. Classical demographic theory shows that the growth rate of a population is dependent on the reproductive value rather than on the net reproductive rate (Caswell, 1989). These two quantities are clearly related, however.

Currently, Georges Bank haddock become vulnerable to the fishery at age 2 but are not fully recruited until age 4 (Table 2). With this partial recruitment vector, F_{st} is 0.519. The graph of λ_e against F (Fig. 1) indicates that if F is held at its 1994 value of about 0.29, the population would be expected to increase at a rate of 6.35% per year. If F is reduced to 0, the stock would be expected to increase at a rate of 18.0% per year. The expected rate of increase when F is below F_{st} is particularly pertinent to cases where stock rebuilding is desired because this analysis allows the de-

termination of how rapidly the stock will be rebuilt under various levels of fishing mortality.

Developing a Leslie matrix representation of harvesting: stochastic case

One of the major challenges facing fishery managers is to determine appropriate reference points for fish populations that show variable recruitment. When one or more elements of the Leslie matrix vary in a stochastic fashion, no general closed-form expressions for the growth rate of the population are available (Tuljapurkar, 1989). Results of theoretical studies of stochastic Leslie matrices are useful, however, in guiding the analysis and interpretation of matrices with entries that vary over time. Two results summarized by Tuljapurkar (1989) that are particularly useful in this analysis are

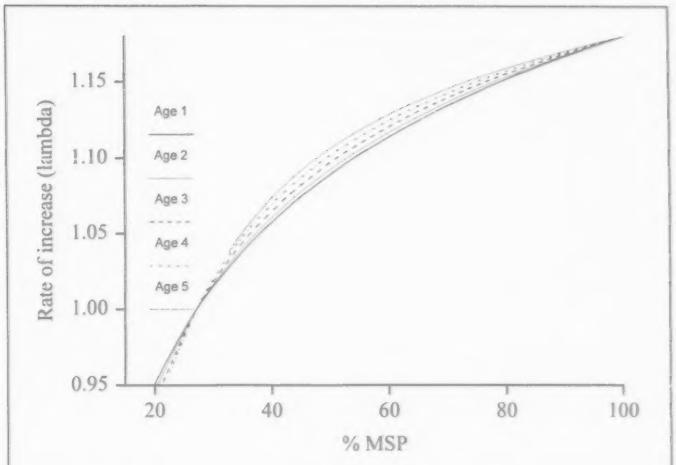

Figure 2

Relationship between rate of population increase (lambda) and percent maximum spawning potential (%MSP) for a range of age at entry.

1 The analog to lambda for deterministic matrices is the mean population growth rate, in contrast to the growth rate of the average population. This is equivalent to the mean rate of change in the logarithm of population size (N).
2 The distribution of projected population size (N) over time tends towards a lognormal distribution when the dynamics are governed by a stochastic matrix.

From these results, maintenance fishing mortality can be defined as the fishing mortality that results in an average population growth rate of 0 (equivalent to λ_e=1). Because this measure is analogous to lambda, I will use the symbol λ for its representation but emphasize that computationally the measures for deterministic and stochastic Leslie matrices differ. An important corollary of the above two results is

3 A population growing deterministically at the mean growth rate does not produce the mean of the population sizes produced in the stochastic representation. Nor does a deterministic matrix composed of the means of the stochastic matrices produce a population with the same dynamics as applying the stochastic matrices.

To illustrate these theoretical results, I performed a simulation of the Georges Bank haddock stock dynamics using a stochastic Leslie matrix. In this case, I focused on the effects of stochastic age-0 survival as represented by R/SSB. I performed this simulation by projecting a starting population forward in time, with the value for R/SSB for each year selected with equal probability from observed values from 1976 to 1994. Five thousand replicates were simulated for a 150-year period.

Results of these simulations are in accord with the theoretical assertion that N_t is distributed lognormally; for times greater than 110 years, the $\ln(N_t)$ did not differ significantly from a normal distribution at α=0.05. It is interesting to note that N_t is lognormally distributed, even though the stochastic element (R/SSB) was not normally or lognormally distributed itself. The lognormal distribution of N_t arises from the fact that N_t is the result of the process of sequential multiplications of random elements (Aitchison and Brown, 1976). When the distribution of population size is plotted over time (Fig. 3), it is clear that the variance increases rapidly. When year-to-year population growth rates (i.e. N_{t+1}/N_t) are computed for individual simulation results, the distribution of growth rates shows an initial transient response for the first 5 years but thereafter settles into a stable distribution from year to year (Fig. 4). Because of the transient dynamics, I began the evaluation of long-term dynamics with year 10.

One of the critical theoretical results is that the growth rate of a population governed by stochastic rates tends towards a single value in the long run. This is what Tuljapurkar (1989) terms the "almost sure population growth rate." When the growth rate is computed over progressively longer intervals, the distribution shows a convergence on nearly the same

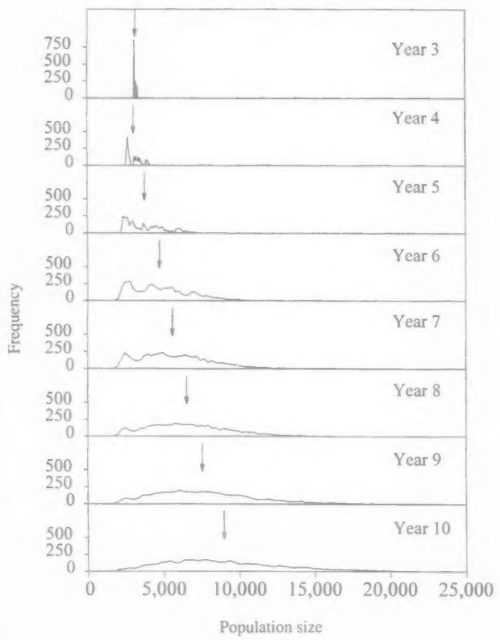

Figure 3

Distribution of population sizes to year 10 for a stochastic projection of the Georges Bank haddock stock. The distribution of population sizes for each time interval is based on 5000 simulations. Arrows indicate mean for each year.

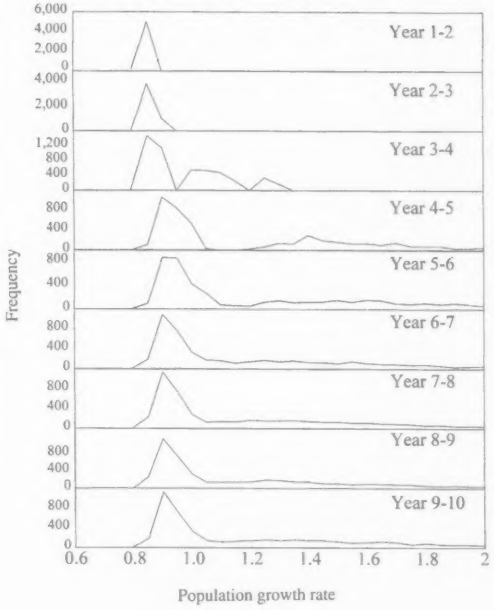

Figure 4

Distribution of annual population growth rates (lambda) to year 10 for a stochastic projection of the Georges Bank Haddock stock. The distribution of growth rates for each time interval is based on 5000 simulations.

mean value (Table 5) with a decreasing variance (Fig. 5). This result has both theoretical and pragmatic implications. Of theoretical importance is the concept that although the variance of projected population abundance increases over time, the variance of the growth rates declines over time. Thus mean population growth rate can be used in defining a maintenance fishing mortality rate. The practical consequence of the above result is that at least two different strategies can be used to compute λ for a stochastic population. One strategy is to project the population for a long period of time (e.g. hundreds of years) to make a precise estimate of the long-term population growth rate. This strategy makes use of the fact that the variance of the long-term population growth declines as the period of projection is lengthened. A problem with this approach, however, is that for projections over a long period of time, population abundance can become so large or small that it cannot be directly represented on a digital computer, causing a numeric overflow or underflow. A preferable strategy is to compute λ for a large number of simulations over a shorter time period (e.g. 150 years). This method avoids the problem of numeric overflow and achieves precision in the estimate of mean λ by having a large number of simulations.

Based on the current partial recruitment vector to the fishery, a fishing mortality of 0.450 (F_{st}) would result in an average population growth rate of zero (Table 6). The fishing mortality that results in a zero growth rate for the mean population size was higher, at 0.517 (Table 6). Interestingly, this is nearly the same as F_{st} computed for the deterministic case by using the mean R/SSB. The estimate of F_{med} with these same data is much lower than F_{st}— only 0.069 (Table 6). When a deterministic Leslie matrix analysis was made with the corresponding median R/SSB, it resulted in an estimate of F_{st} that was nearly the same as F_{med} (Table 6). This finding illustrates the basic connection between these methods when they are operating on the same inputs. As an additional comparison, I computed SSB/R as the ratio ΣSSB/ΣR instead of the mean (or median) of the individual year ratios. This method is based on sampling theory that suggests that the ratio of the sums is a less biased estima-

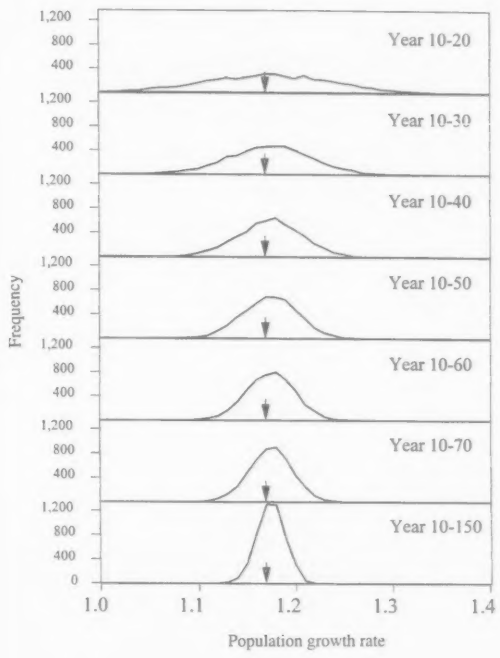

Figure 5

Distribution of population growth rates over various time scales for a stochastic projection of the Georges Bank haddock stock. The distribution of growth rates for each time interval is based on 5000 simulations. Arrows indicate mean for each year.

Table 5

Mean and coefficient of variation (CV) of 5000 simulations of long run population growth rate for Georges Bank Haddock without fishing.

Time interval	Mean	CV
Year 10–20	0.1552	37.02
Year 10–30	0.1555	23.28
Year 10–40	0.1558	18.03
Year 10–50	0.1559	15.23
Year 10–60	0.1559	13.34
Year 10–70	0.1560	12.07
Year 10–150	0.1563	7.60

Table 6

Comparison of F_{st} with deterministic analyses, stochastic analyses, and with %MSP calculations.

Type of simulation	Recruitment input	F_{st}
Deterministic	Mean R/SSB	0.519
Deterministic	Median R/SSB	0.070
Stochastic (mean of rates)	Uniform probability	0.450
Stochastic (mean of populations)	Uniform probability	0.517
%MSP	Mean SSB/R	0.519
%MSP	Median SSB/R	0.069
%MSP	$SSB/R = \Sigma SSB/\Sigma R$	0.449

tor of the "true" ratio than is the mean of the individual ratios (e.g. Cochran, 1977). The value for SSB/R obtained with this method was 2.685, and when used in place of the median SSB/R in the F_{med} calculations, resulted in an estimated reference point of 0.449—essentially the same as in the stochastic simulation where the mean population growth rate was zero (Table 6).

Discussion

The primary purpose of this paper was to demonstrate how the Leslie matrix can be used to compute a reference point for harvested populations and to contrast this method with the SSB/R method currently in use. One of the major findings was that the SSB/R method and the Leslie matrix approach produce similar estimates of sustainable fishing mortality when they are based on the same inputs. This is not surprising given the similarity between reproductive value, on which the Leslie matrix is based, and lifetime spawning stock biomass per recruit which the SSB/R approach uses.

Although the two methods produce similar estimates of sustainable fishing mortality, the Leslie matrix approach is preferable because of the additional direct information it provides regarding the population response to fishing at levels different from the reference point. Furthermore, when population growth rate is different from zero, equal levels of SSB/R do not result in the same population growth rate for different partial recruitment vectors. These differences are small, however, in relation to changes in population growth rate owing to changes in fishing mortality.

Given the various approaches illustrated (e.g. deterministic vs. stochastic), the basic question is what method to use. On the basis of theoretical advances in the population dynamics literature and results presented here, I recommend the use of a stochastic analysis where the mean of the population growth rates is used as the "best" measure of growth for harvested populations. A stochastic analysis is preferable be-

cause it can fully represent the information contained in the distribution of observed R/SSB values. Also, a stochastic simulation can be used to provide a measure of the uncertainty associated with estimates of the biological reference point. The use of the mean population growth rates instead of the mean of the population sizes is justified on theoretical grounds (Tuljapurkar, 1989). As shown in Table 6, use of the mean R/SSB in a deterministic analysis or the rate of growth of the mean population size in a stochastic simulation tends to result in higher estimates of sustainable fishing mortality (or, alternatively, a higher estimate of population growth rate for a given fishing mortality rate) than does the stochastic simulation where the mean of population growth rates are used as the output. I feel that the reason for this difference hinges on the distinction between projections and forecasts (Caswell, 1989) . If we view the long-term simulation results as forecasts, this implies that we could use the distribution of population size (and the mean of the distribution) as the best estimate of the future state of the population. In these simulations, however, the mean population size is strongly influenced by the very high values that occur in the right-hand tail of the lognormal distribution. These estimates are far above what has ever been observed for this stock, and are probably not biologically realistic. As such, they should not be treated as true forecasts of the future population. In contrast, if we view the analysis as a projection, the goal is not to forecast future population size but rather to use the results to determine the population growth rate that is represented by the current Leslie matrix. By knowing the current population growth rate, it is possible to find the fishing mortality rate that maintains an expected value for population growth of zero, which would result in a statistically stationary population.

In a stochastic setting, the entire distribution of R/SSB ratios is used to portray the reproductive success for the stock. When a deterministic analysis is desired, however, it is necessary to choose among several possible measures of central tendency for the R/SSB ratio. Sissenwine and Shepherd (1987) advocated the use of the median R/SSB ratio as a way of robustly portraying the reproductive success of a stock. Their rationale was that the frequency of relatively poorer recruitment is balanced by years of better recruitment when the median R/SSB is used. Table 6 illustrates, however, that using the median of the observed R/SSB ratios can result in estimates of sustainable fishing mortality that are substantially different from the stochastic simulations, which are arguably the best to represent the population's dynamics. The use of the mean of the observed R/SSB ratio as the measure of central tendency can likewise result in estimates of sustainable fishing mortality that differ from the standard set by stochastic simulations. The primary reason for this difference is that use of a mean of the observed ratios is biased high in relation to the preferred estimator of the ratio (in this case the sum of recruitment over the sum of spawning stock biomass; Cochran, 1977). As such, the use of the mean of the observed R/SSB values can also be inaccurate. Among the measures presented here, the estimator Σrecruitment/Σspawning stock biomass should be used as the measure of central tendency for the R/SSB ratio in deterministic analyses. The use of this measure results in point estimates of sustainable fishing mortality that are essentially the same as a full stochastic analysis.

Although the Leslie matrix is a useful tool to portray the dynamics of harvested populations and to determine appropriate reference points, several issues arise that are of considerable practical importance. As alluded to earlier, a significant challenge is how to determine what is an appropriate distribution for the R/SSB ratio. Because of the variability in R/SSB over time and the occurrence of occasional large year classes, it is very difficult to determine what time frame is representative of the present. Although the answer to this question is beyond the scope of this paper, I feel that the best approach is to plot the mean R/SSB ratio over progressively longer time periods back from the present to determine if there are any temporal trends or epochs in the data set. The analyst should then use his or her judgment based on other biological information over time (such as stock size, mean weight per individual at age, and maturation schedule) to determine an appropriate period to use as the basis for stochastic simulations. It is important to emphasize that the dilemma of choosing a representative time period is not unique to analyses in which the Leslie matrix is used, and the same problem arises for computing any biological reference point.

In addition to the difficulty of determining what is a representative time period for the present population, a fundamental question is how to represent the dynamics of populations with a density-dependent stock-recruitment relationship. In principle, this can be approached by altering the R/SSB ratio as a function of stock size (e.g. Quinn and Szarzi, 1993). Although I agree with Quinn and Szarzi's (1993) approach, the challenge of accurately specifying the distribution of R/SSB ratios at different stock sizes is even greater than specifying the current distribution.

As a final comment, biological reference points for fish populations are not necessarily targets for fishery management (Mace, 1994), nor are they inviolate boundaries that may not be crossed. Rather, they are most useful as a means of comparing the consequences

of different choices among fishery management options. For example, it is appropriate to allow fishing mortality to exceed the biological reference point if the goal is to reduce an overly abundant fish stock. Likewise, they can be useful in projecting the likely growth of a population under more restrictive fishery management measures. In the end, however, they may be most useful as a reminder and a warning that there are limits to the productive capacity of fish population and that if we consistently exceed their limits, population declines are almost certain to occur (Francis, 1997; Myers, 1997).

Acknowledgments

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Biological effects of shellfish harvesting on oyster reefs: resolving a fishery conflict by ecological experimentation

Hunter S. Lenihan
Beaufort Laboratory
National Marine Fisheries Service, NOAA
101 Piver's Island Road, Beaufort, North Carolina 28516
Present address: Institute of Marine Sciences
University of North Carolina at Chapel Hill,
3431 Arendell St., Morehead City, North Carolina
E-mail address: hsleniha@email.unc.edu

Fiorenza Micheli
National Center for Ecological Analysis and Synthesis
Santa Barbara, California 93101

Abstract.—We conducted a large-scale field experiment to test whether clam and oyster harvesting applied alone and in combination on intertidal oyster reefs have impacts on resident shellfish populations. This experiment was conducted to resolve a long-standing conflict between oyster (*Crassostrea virginica* (Gmelin, 1791)) and clam (*Mercenaria mercenaria* (Linneaus, 1758)) fishermen who contend that the other fishery causes high rates of mortality to their respective species. Intertidal oyster reefs located in two estuarine creeks near Wilmington, North Carolina, were harvested for clams only, oysters only, both clams and oysters, or were left undisturbed as controls. Experimental harvesting was conducted over a one-year period by a professional shellfisherman who used realistic fishing techniques (clam rakes and oyster tongs), intensity, and frequency. Harvesting impact on hard clam and oyster populations was assessed by sampling naturally occurring oysters before and after harvesting, and sampling both naturally occurring clams (all size classes) and transplanted, hatchery-raised clams (20–37 mm in length) after harvesting. Clam and oyster harvesting had obvious negative effects on populations of oysters. There was a substantial decrease in the number of live oysters on clam-harvested and oyster-harvested reefs compared with unharvested, control reefs. Clam and oyster harvesting, applied together, reduced oyster densities and killed unharvested oysters at a level similar to that caused by each type of harvesting applied separately. The effects of the shellfish harvesting on populations of hard clams varied between the two sites (i.e. creeks). In both creeks, clam harvesting, alone and combined with oyster harvesting, significantly decreased the number of live, naturally occurring clams. Oyster harvesting alone decreased the number of live, naturally occurring clams only at one site. Clam harvesting also decreased the number of live, transplanted clams on reefs, but there was no effect of oyster harvesting, because the transplanted clams were juveniles too small to be harvested with oyster tongs. Overall, the combined effect of both types of harvesting applied together did not have a negative synergistic effect on clam and oyster populations. Consequently, both clamming and oyster harvesting should be permitted on some reefs, but maintaining large populations of oysters and clams on intertidal oyster reefs will require protection of some reefs from both types of harvesting.

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Marine fisheries are an important source of employment and protein for humans but can negatively affect marine organisms and ecosystems (Dayton et al., 1995; Engel and Kvitek, 1995; Botsford et al., 1997). The most obvious negative ecological effects of fishing result from over-harvesting of target species, incidental mortality of nontarget species ("bycatch"), and fishery-related disturbances to marine habitat (FAO, 1993; Dayton et al., 1995). Of course, fisheries over-exploitation and habitat destruction also threaten the sustainability of the fishing industry. At present, 44% of the worlds fish stocks are fully to heavily exploited, and 22% are overexploited or depleted, indicating most fisheries are not managed for long-term sustainability (Botsford et al., 1997). The degradation and destruction of marine biogenic habitat (e.g. coral reefs, seagrass beds, mangrove forests, and oyster reefs) by dredging, trawling, use of explosives, and poisoning reduces fishery production by removing habitat essential for the recruitment, growth, and survival of fishery and prey organisms (Winslow 1881, a and b; Peterson et al., 1987; Norse, 1993; Rothschild et al., 1994; NRC, 1995; Lenihan and Peterson, 1998). The sustainability of a fishery is often threatened when competing fisheries exploit a common resource or negatively impact a commonly used habitat. For example, when the bycatch of one fishery is within a food web supporting another fishery (West and Gordon, 1994), or when a fishery destroys habitat important to the life history of other fishery species (Russ and Alcala, 1996), heated political battles arise and the livelihood of many people may be lost. Resolving such fishery conflicts has important ecological and economic consequences and is of major concern to fisheries managers and ecologists worldwide (McAllister and Peterman, 1992). This paper presents the results of an experimental analysis of whether two economically valuable fisheries conflict and provides management recommendations to resolve the conflict.

High productivity of fishery stocks in estuaries and shallow water coastal habitats often induces intense exploitation of a common species or habitat by multiple, potentially conflicting fisheries (Peterson et al., 1987). In many estuaries along the Atlantic coast of the USA, intertidal oyster reefs are harvested for hard clams (*Mercenaria mercenaria*) year round,

and for oysters (*Crassostrea virginica*) in the fall and winter (i.e. October–March). In recent years, clam and oyster (i.e. "shellfish") harvesting on oyster reefs has led to conflict between the two fisheries, and between fishermen and habitat managers over the issue of habitat degradation, especially in the southeastern United States (e.g. Frankenberg[1]; Noble[2]). Oyster fishermen claim that the harvesting of clams from intertidal oyster reefs decreases resident oyster populations, and vice-versa, because each type of fishing kills or removes the other species. Rakes and hand tongs used for the two types of shellfishing appear to increase the mortality of the sessile reef animals by burying them beneath sediments, fracturing their shells, or causing other physical damage (Noble[2]). In addition, bodies of dead and wounded animals left behind may attract scavengers and predators, thereby increasing predation intensity on healthy live animals (Dayton et al., 1995). Habitat and fishery managers are concerned that the physical destruction of oyster reefs caused by shellfishing will negatively affect many other fishery organisms that recruit to and utilize oyster reef habitat, including many species of fishes (Breitburg et al. 1995, Lenihan et al., 1998, Luckenbach et al., 1998) and the blue crab (*Callinectes sapidus* (Rathbun)) (Bahr and Lanier, 1981; Zimmerman et al., 1989; Lenihan et al., 1998; Micheli and Peterson, 1999). Shellfishing also reduces the overall size of reefs because shell material is broken or removed along with the target species (Lenihan and Peterson, 1998; Coen[3]). Reducing the size of reefs is thought to decrease the abundance of clams because less habitat is available for adults and recruits (Arnold, 1984; Sponaugle and Lawton, 1990; Peterson et al., 1995). Decreasing the size (i.e. height) of oyster reefs also reduces oyster production because flow speed over reefs diminishes, causing sediment to accumulate and oyster growth and survival to decrease (Lenihan, 1999; Lenihan et al., 1999). In contrast to the negative effects of shellfish harvesting, many fishermen claim that "turning over" the shell matrix of oyster reefs during harvesting improves clam and oyster production because it removes accumulated sediment. In North Carolina and many other Atlantic coast states, both types of shellfishing are allowed on reefs and conflicts between the fisheries continue (e.g. Frankenberg[1]; Marshall[4]). Whether oyster harvesting, clam harvesting, or both types of fishing activities together have negative impacts on shellfish populations of intertidal oyster reefs is a matter of opinion and has not been tested experimentally.

A comparison of the biological impact of various fishing practices by using large-scale field experiments is an efficient method of resolving many fishery-related conflicts (McAllister and Peterman, 1992) and is an important component of adaptive management strategies (Walters, 1986). Such experiments are usually designed so that replicate areas (i.e. treatments) are fished, by using each technique separately and by using a combination of techniques, while other areas (i.e. controls) are closed to fishing. For these experiments to be meaningful, they must be conducted on realistic temporal and spatial scales, and the fishing treatments must be applied through the actual fishery (McAllister and Peterman, 1992). The success of such experiments also depends heavily on close working relationships among fishermen, fishery ecologists, and fishery managers (Grumbine, 1997). For adaptive management, the results of initial (i.e. "prototype") experiments are used to design new management strategies that are subsequently tested on even larger temporal and spatial scales. Such adaptive management strategies and the use of experimental approaches are often discussed in fisheries management, but in reality are rarely attempted (e.g. Walters, 1986; Botsford et al., 1997).

We conducted a large-scale field experiment to test the effects of hard clam and oyster harvesting, separately and in combination, on oyster and hard clam populations living on intertidal oyster reefs in North Carolina. Specifically, we tested whether 1) the density of live and dead oysters varied among oyster reefs that were harvested for clams, harvested for oysters, harvested for clams and oysters, or were unharvested; 2) the density of live and dead clams varied among oyster reefs subjected to the same four harvesting treatments; and 3) the joint application of both shellfish harvesting practices has a synergistic (i.e. a multiplicative) effect on each species. If applying both types of harvesting to the same reefs enhances potential negative effects of each harvesting type, a possible management option would be to allow clam and oyster harvesting only on separate reefs. This experiment was designed and conducted with the combined effort of a clam and oyster fisherman,[5] ecologists,[6] and habitat

[1] Frankenberg, D. 1995. Report of North Carolina Blue Ribbon Advisory Council on oysters. North Carolina Department of Environmental Health, and Natural Resources, Raleigh, NC, 101 p.

[2] Noble, E. B. 1995. Destruction of oyster rocks by individuals taking clams by legal hand harvest methods. Report of the North Carolina Division of Marine Fisheries, Morehead City, NC, 11 p.

[3] Coen, L. D. 1995. A review of the potential impacts of mechanical harvesting on subtidal and intertidal shellfish resources. A report prepared by the South Carolina Department of Natural Resources, Marine Resources Research Institute, SC, 111 p.

[4] Marshall, M. 1996. North Carolina Division of Marine Fisheries, 3431 Arendell St., Morehead City, NC, 28557. Personal commun.

[5] Cummings, R. A. 1996. For address contact H. S. Lenihan, Institute of Marine Sciences, 3431 Arendell, Morehead City, NC 28557. Personal commun.

[6] Peterson, C. H., and H. C. Summerson. 1997. Institute of Marine Sciences, 3431 Morehead City, NC 28557.

managers,[7] and is a prototype experiment for adaptive management of shellfisheries in southeastern North America.

Methods

Study sites

The intertidal oyster reefs used in this study were located in two large tidal creeks, Pages and Whiskey Creeks, situated on the Intercoastal Waterway near Masonborough Inlet, Wilmington, North Carolina. The two creeks consisted of well-flushed sandy to muddy bottom tidal channels 0–2 m in water depth. Channels in each creek were separated by small to large patches of marsh (*Spartina alterniflora*) habitat surrounded by oyster reefs created by *Crassostrea virginica*. The two creeks were chosen because they have been permanently closed to fishing for about the last ten years owing to high coliform bacteria counts caused by the seepage of septic tanks from surrounding homes. Both creeks are highly productive, supporting large populations of fishes, birds, crabs, clams, and oysters. Tides in each creek are predominantly M-2 lunar tides, and the tidal range is 1–2 m in both creeks. Four large oyster reefs (9–13 m wide × 45–55 m long), each containing relatively high densities of oysters and hard clams, were chosen in each creek. The reefs were situated 150–200 m from the mouth of each creek. The salinity near the experimental reefs was 22–34 psu throughout the course of the experiment and water temperature was 3–30°C.

Three to five permanent 6-m long × 1-m wide transects were established haphazardly on each oyster reef by using PVC poles with rebar anchors between 1–14 June 1996. A total of sixteen transects were established in each creek at approximately 0.5 m above the mean low tide level. The sixteen transects provided a total of four replicates of each of the following four harvest treatments: clam harvesting only, oyster harvesting only, clam harvesting and oyster harvesting combined, and no harvesting. Reefs and transects were located in areas where disturbances caused by shellfishing, boat traffic, or other human activities did not normally occur. We found no evidence suggesting that experimental reefs were physically or chemically disturbed throughout the course of the experiment.

Sampling of clams and oysters

The density of live and dead oysters on each experimental oyster reef was measured between 5 and 10 July 1996 before harvest treatments were applied. Oyster density was measured by counting (but not removing) oysters in three 0.25-m^2 permanent plots established in each of the sixteen transects in each creek. Plots were established by stretching a measuring tape between the two PVC poles marking each transect and by placing a PVC quadrat at 0.5-, 2.5-, and 3.5-m distance. All live and dead oysters were counted in each quadrat. The density of naturally colonized clams was not determined prior to the application of harvest treatments to avoid disturbing the reefs and potentially influencing the condition of the remaining clams and oysters. Instead, between 5 and 10 July, 16 hatchery-raised clams provided by ARC, Inc. of Atlantic, North Carolina, were placed in each of three 1-m^2 quadrats established within each 6-m transect. This introduction of transplanted clams was done to assure that enough clams were present for the experiment. The 1-m^2 quadrats were also placed at 0.5, 2.5, and 3.5 m distance along the transects. Before being transplanted, hatchery clams were dyed in Alizarin red dye in order to distinguish them from natural clam populations (Peterson et al., 1995). Of the 16 clams in each plot, eight were 20–25 mm in length, and eight were 27–32 mm in length.

Between October 1996 and March 1997, oysters were harvested with hand tongs during low tides within the 1) oyster harvesting and 2) clam and oyster harvesting treatments. Oysters were harvested for the same total period of time (3.75–4.0 h/transect) along the entire length of each transect. From August 1996 to May 1997, clams were harvested during low tides with clam rakes and clam tongs from the 1) clam harvesting and 2) clam and oyster harvesting treatments, and for approximately the same total period of time (i.e. 3.75–4.0 h/transect). The total number of naturally occurring and transplanted clams and oysters removed during the harvest was recorded. All harvesting was conducted by R. A. Cummings, a professional shellfisherman.

The density of live and dead clams and oysters remaining on experimental reefs was sampled 10–23 July 1997, after termination of the harvesting treatments. Clams and oysters were sampled several months after the last clam harvesting in May so that any potential long-term effects of harvesting were realized. For example, unharvested clams and oysters remaining on reefs may have been injured during harvesting and died after several weeks. Oysters were sampled by placing a measuring tape along the transects and counting all oysters within the three 0.25-m^2 quadrats at 0.5-, 2.5-, and 3.5-m distance along each transect. Clams were sampled by digging up the top 25 cm of sediment from each 1-m^2 sampling plot. The sediment was then passed through a 1-mm sieve to remove all clams.

[7] Carpenter, R., and M. Marshall. 1996. North Carolina Division of Marine Fisheries, 3431 Arendell St., Morehead City, NC 28557.

Table 1

Mean square errors (MS), *F* ratios, and corresponding significance levels (*P*) of two-way fixed factor ANOVAs comparing densities of live and dead oysters, and proportions of dead oysters (per 0.25 m^2) among intertidal oyster reefs before application of experimental harvest treatments (sampled 5–10 July 1996). The main factors in ANOVAs were creeks (Pages and Whiskey Creeks) and harvest treatment (clamming, oystering, both, and neither).

		Live			Dead			Proportion dead		
Source	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Creek (C)	1	277.50	6.17	0.02	378.10	34.58	0.0001	0.03	30.11	0.0001
Harvest treatment (H)	3	31.03	0.69	0.57	0.41	0.04	0.99	0.001	0.48	0.70
C × H	3	31.74	0.71	0.56	12.27	1.12	0.36	0.001	1.19	0.33
Residual	24	45.01			10.93			0.001		

Statistical analyses

The density of live and dead oysters, and the proportion of the total number of oysters that were found dead before harvesting, were compared among treatments by using two-way, fixed factor analysis of variance (ANOVA) tests. The two main factors in the ANOVAs were creeks (Pages and Whiskey Creeks) and harvest treatments (clam harvesting, oyster harvesting, both, or neither). The same ANOVA model was used to test for differences in 1) the density of live and dead oysters, and the proportion of dead oysters (i.e. number of dead oysters/live + dead oysters) after harvesting, 2) the density of live and dead, and proportion of dead, naturally occurring clams after harvesting, and 3) the density of live and dead, and number of missing transplanted, hatchery-raised juvenile clams after harvesting. Before all ANOVAs, homogeneity of variances was tested by using Cochran's test (at α=0.05). When variances were heterogeneous, data were log transformed and homogeneity was retested. After ANOVAs, *post hoc* tests for differences among treatment means were conducted with Student-Newman-Keuls method (SNK) tests (at α=0.05).

Results

In July 1996, prior to the application of experimental harvests, the number of live and dead oysters (those observed with naked eye; >1 mm in length) and the proportion of dead oysters in experimental plots did not vary with the interaction of creeks and harvest treatment (ANOVA, *creek × harvest treatment interaction*, P=0.33–0.56; Table 1), nor among harvest treatments (P=0.57–0.99), but differed significantly between creeks (P=0.02–0.0001). Whiskey Creek had greater numbers of live and dead oysters and proportion of dead oysters than Pages Creek (Figs. 1 and 2).

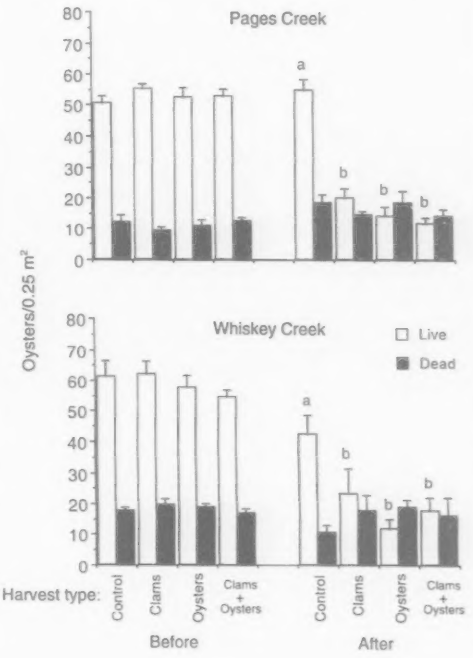

Figure 1

Mean density of live and dead oysters (>1 mm in length) before (5–10 July 1996) and after (10–23 July 1997) application of experimental harvest treatments in Pages and Whiskey Creeks, NC. Data are means and one standard error (n=4) of counts taken within 0.25-m^2 quadrats. Results of SNK *post hoc* comparisons are illustrated with letters above bars (a>b at P<0.05). Separate ANOVAs and SNK tests were used to compare numbers of live and dead oysters both before and after harvesting.

Experimental clam harvesting conducted from August 1996 to May 1997 removed only hard clams from experimental plots (Table 2). In contrast, a few clams

were caught in oyster tongs during oyster harvesting, which was conducted from October 1996 to March 1997. In both creeks, two to three times the number of clams were harvested during clam harvesting treatments than during oyster harvesting. Similar numbers of clams were removed from reefs in the clam harvesting and the combined clam and oyster harvesting treatments. Similar numbers of oysters were removed from plots harvested for oysters only and from those harvested for both oysters and clams (Table 2). According to visual observations, both types of harvesting inflicted obvious wounds (holes and cracks) to the shells of oysters (range: 5–13 individuals within each plot) that were not removed by harvesting.

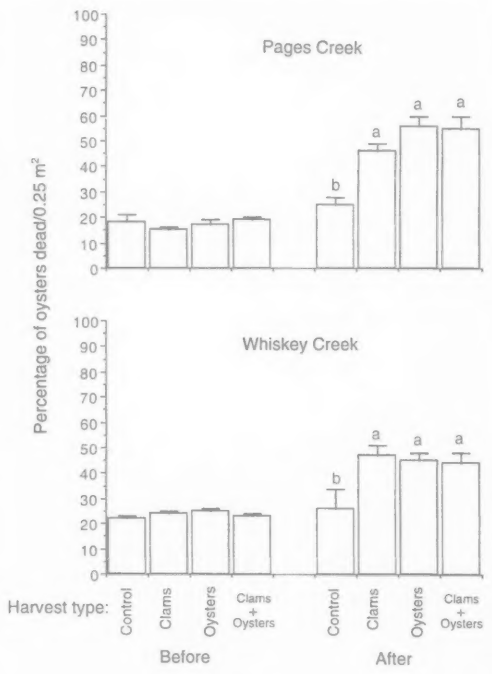

Figure 2

Mean percentage of oysters found dead before (5–10 July 1996) and after (10–23 July 1997) application of experimental harvest treatments in Pages and Whiskey Creeks, NC. Data are means and one standard error (n=4) of counts taken within 0.25-m² quadrats. Results of SNK *post hoc* comparisons are illustrated with letters above bars (a>b at P<0.05). Separate ANOVAs and SNK tests were used to compare numbers of dead oysters before and after harvesting.

In July 1997, after experimental clam and oyster harvesting, the density of live and dead oysters, and the proportion of dead oysters did not vary with the interaction of creeks and harvest treatment (ANOVA; *creek* × *harvest treatment interaction*, P=0.23-0.44; Table 3). There was also no significant difference in the density of live and dead oysters and the proportion of dead oysters between the two creeks (P=0.16–0.65; Table 3). In contrast, there was a highly significant effect of harvest treatment on the density of live oysters and the proportion of oysters found dead (P=0.0001; Table 3). At both sites, plots harvested for clams, oysters, or both had 2–4.5 times lower densities of live oysters and 2–2.5 times higher proportions of dead oysters than did unharvested control plots (SNK, P<0.05 for both contrasts; Figs. 1 and 2). There were no differences in the number of dead oysters among harvest treatments.

In July 1997, after experimental harvesting, the density of live, naturally occurring hard clams varied with the interaction of creeks and harvest treatments (ANOVA, *creek* × *harvest treatment interaction*, P=0.015; Table 4). At Pages Creek, there were greater numbers of live, naturally occurring clams in control reefs than in plots harvested for clams, oysters, or both (SNK; P<0.05; Fig. 3). At Whiskey Creek, there were more live, naturally occurring clams in both control and oyster-harvested plots than in plots harvested for clams and for both species (SNK, P<0.05 for both contrasts; Fig. 3). The number and proportion of dead, naturally occurring clams found in July 1997 did not vary with the interaction of creeks and harvest treatment (ANOVA, *creek* × *harvest treatment interaction*, P=0.09–0.87; Table 4), or between creeks (P=0.16–0.10; Table 4). There was also no significant effect of harvest treatment on the density of dead, naturally occurring clams (P=0.17; Table 4). However, there was a significant effect of harvest treatment on the pro-

Table 2

Mean number of clams and oysters removed from intertidal oyster reefs during experimental harvesting. Reefs were harvested for clams (clamming), oysters (oystering), both (clamming and oystering), or neither (controls). Transplanted, hatchery-raised clams were not removed during harvesting.

	Pages Creek		Whiskey Creek	
Harvest treatments	Clams	Oysters	Clams	Oysters
Controls	0	0	0	0
Clamming	3.47 ±1.1	0	11.77 ±7.37	0
Oystering	1.15 ±0.22	69.20 ±9.20	5.05 ±2.89	43.27 ±14.10
Clamming and oystering	3.46 ±0.75	89.40 ±58.32	12.59 ±5.84	34.97 ±8.26

Table 3

Mean square errors (MS), *F* ratios, and corresponding significance levels of (*P*) two-way fixed factor ANOVAs comparing densities of live and dead oysters, and proportions of dead oysters (per 0.25 m^2) among intertidal oyster reefs after application of experimental harvest treatments (10–23 July 1997). The main factors in ANOVAs were creeks (Pages and Whiskey Creeks) and harvest treatment (clamming, oystering, both, and neither).

		Live			Dead			Proportion dead		
Source	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Creek (C)	1	18.45	0.21	0.65	55.52	1.02	0.32	0.02	2.10	0.16
Harvest treatment (H)	3	2192.00	25.25	0.0001	4.45	0.08	0.97	0.12	13.86	0.0001
C × H	3	132.90	1.53	0.23	67.70	1.24	0.32	0.01	0.93	0.44
Residual	24	86.82			54.54			0.01		

Table 4

Mean square erros (MS), *F* ratios, and corresponding significance levels (*P*) of 2-way fixed factor ANOVAs comparing densities of live and dead hard clams, and proportions of dead clams (per 1.0 m^2) among intertidal oyster reefs after application of harvest treatments (10–23 July 1997) The main factors in ANOVAs were creeks (Pages and Whiskey Creeks) and harvest treatment (clamming, oystering, both, and neither).

		Live			Dead			Proportion dead		
Source	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Creek (C)	1	277.5	6.17	0.02	378.1	34.58	0.0001	0.03	30.11	0.0001
Creek (C)	1	0.13	0.03	0.85	10.7	2.15	0.16	0.07	2.94	0.10
Harvest treatment (H)	3	36.55	10.01	0.0002	8.97	1.80	0.17	0.16	6.19	0.003
C × H	3	15.60	4.27	0.015	12.28	2.47	0.09	0.01	0.23	0.87
Residual	24	3.65			4.98			0.03		

portion of dead, naturally occurring clams (P=0.003; Table 4). The proportion of dead clams in both creeks was much higher on harvested than on unharvested (i.e. control) reefs (SNK, P<0.05 for both contrasts; Fig. 4) but was similar among the three harvest treatments (SNK, P>0.05 for both contrasts; Fig. 4).

After harvesting, the density of live and dead hatchery-raised clams transplanted to reefs at the beginning of the experiment tended to vary with the interaction of creeks and harvest treatment, although not significantly (ANOVA, creek × harvest treatment interaction, P=0.07-0.08; Table 5). However, the density of live transplanted clams varied between creeks (P=0.03; Table 5) and among harvest treatments (P=0.04; Table 5). More transplanted clams were recovered alive in Pages Creek (mean ± 1SD: 3.21 ±1.62/m^2) than in Whiskey Creek (2.22 ±1.45/m^2). Fewer live transplanted clams were recovered from clam-harvested plots than from control plots in both creeks (SNK, P the interaction of <0.05; Fig. 5). The number of dead transplanted clams found after harvesting also varied between creeks (Pages Creek>Whiskey Creek; P=0.0001; Table 5) but did not vary significantly with harvest treatment (P=0.10; Table 5). At Pages Creek, there was a slight trend for greater mortality of transplanted clams on clam-harvested and clam- and oyster-harvested plots than in oyster-harvested and control plots only (Fig. 5). Most transplanted clams placed on reefs at the beginning of the experiment were not found at the end of the experiment ("missing" clams; Fig. 5). The number of missing transplanted clams differed with the interaction of creeks and harvest treatment (ANOVA, *creek × harvest treatment interaction*, P=0.03; Table 5) because fewer clams were recovered in our census in the oyster-harvested plots than in clam-harvested plots at Whiskey Creek only (SNK; P<0.05; Fig. 5).

Discussion

Our results clearly demonstrate that both clam and oyster harvesting significantly reduce oyster popula-

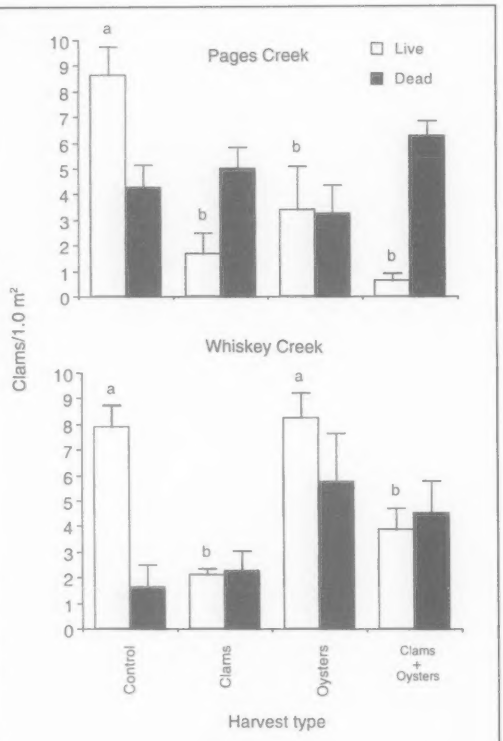

Figure 3

Mean density of live and dead naturally-occurring hard clams found after (10–23 July 1997) application of experimental harvest treatments in Pages and Whiskey Creeks, NC. Data are means and one standard error (n=4) of counts taken within 1.0-m² quadrats. Results of SNK *post hoc* comparisons are illustrated with letters above bars (a>b at P<0.05). Separate ANOVAs and SNK tests were used to compare numbers of live and dead clams.

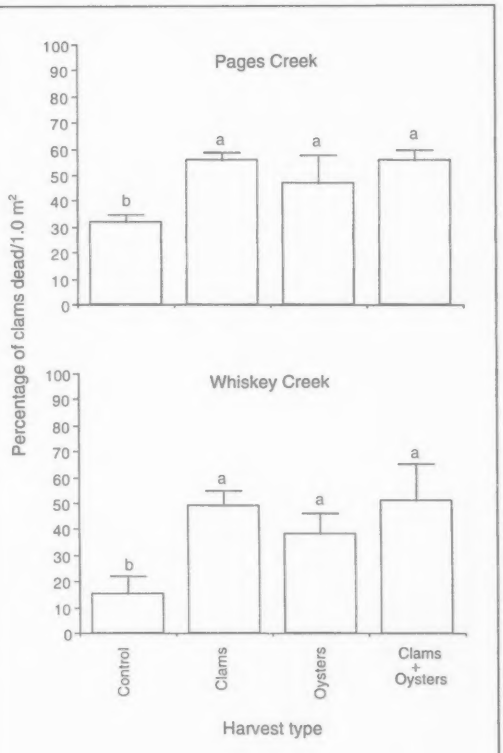

Figure 4

Mean percentage of naturally-occurring hard clams found dead after (10–23 July 1997) application of experimental harvest treatments in Pages and Whiskey Creeks, NC. Data are means and one standard error (n=4) of counts taken within 1.0-m² quadrats. Results of SNK *post hoc* comparisons are illustrated with letters above bars (a>b at P<0.05).

Table 5

Mean square errors (MS), F ratios, and corresponding significance levels (P) of 2-way fixed factor ANOVAs comparing numbers of live, dead, and missing hatchery-raised hard clams (per 1.0 m²) among intertidal oyster reefs after application of harvest treatments (sampled 10–23 July 1997). Before application of harvest treatments, hatchery-raised juvenile clams were placed at equal densities (16 clams/m²) on each reef. The main factors in ANOVAs were creeks (Pages and Whiskey Creeks) and harvest treatment (clamming, oystering, both, and neither).

		Live			Dead			Proportion dead		
Source	df	MS	F	P	MS	F	P	MS	F	P
Creek (C)	1	0.14	5.48	0.03	14.45	26.54	0.0001	41.63	19.33	0.0002
Harvest treatment (H)	3	0.08	3.24	0.04	1.28	2.35	0.10	2.03	0.94	0.44
C × H	3	0.07	2.67	0.07	1.40	2.58	0.08	7.65	3.55	0.03
Residual	24	0.03			0.54			2.15		

tions on intertidal oyster reefs. Both types of shellfish harvesting, applied separately or together, reduced the densities of live oysters by 50–80% compared with densities at unharvested reefs. Surprisingly, there was no difference among the effects of clam harvesting only, oyster harvesting only, and clam and oyster harvesting combined on the density of live oysters. We expected oyster harvesting to reduce oyster populations more than clam harvesting because oyster harvesting removes oysters whereas clam harvesting targets clams only (see Table 2). We do not know the effect of clam and oyster harvesting on oysters <1 mm in length; therefore further experiments should be conducted to determine their fate. Results of our experiment show conclusively that the density of live, adult oysters was significantly reduced on reefs that were harvested for clams only (Fig. 1). Therefore, clam harvesting has important negative effects on oysters, most likely through increased oyster mortality.

We did not investigate the specific mechanisms underlying the negative effect of clam harvesting on oyster populations, but observations made during experimental harvesting indicated that clamming with rakes killed oysters in two ways. First, during the process of clamming oyster shells were cracked or punctured (senior author, personal obs.) Severely wounded oysters probably died. Oysters were also indirectly killed during clamming when they were buried or smothered beneath sediments that were removed in the process of digging for buried clams (senior author, personal obs.). Another potential, but unobserved, mechanism potentially leading to enhanced oyster mortality during clamming was that predators (e.g. blue crab and the sheepshead fish, *Archosargus probatochephalus*) were attracted to the reefs by wounded oysters and by sediment disruption, thereby enhancing predation intensity on oysters (e.g. Dayton et al., 1995). It did not appear that oysters were spread around on the experimental reefs by clam harvesting, thus reducing their densities in sampling plots.

Effects of clam and oyster harvesting on naturally occurring populations of hard clams were less clear than effects of clam and oyster harvesting on oysters. Clam harvesting, both alone and in combination with oyster harvesting, decreased densities of live clams by 50–90% compared with unharvested reefs. This result was expected because clam harvesting removes large numbers of clams (see Table 2). Because clams are motile, it is possible that some clams emigrated from sampling plots following the harvest disturbance, thereby accounting for some reduction in clam density. However, this movement is unlikely to have accounted for a large proportion of the reduction in clam densities because the sampling plots covered much of the area on reefs inhabited by clams. Oyster harvesting

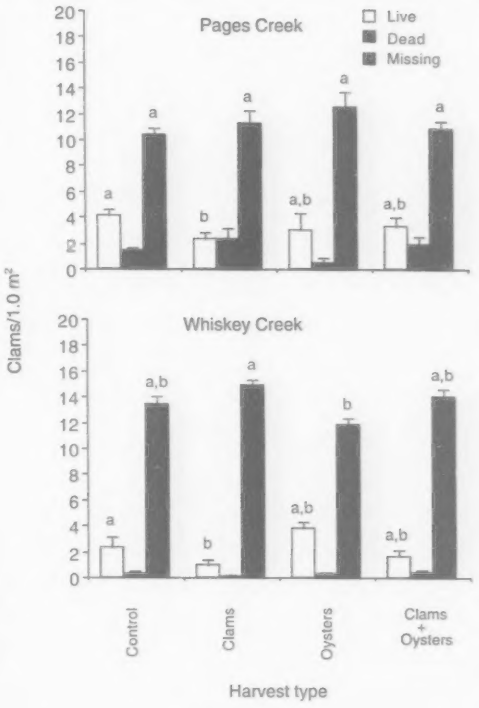

Figure 5

Mean density of live, dead, and missing hatchery-raised hard clams after (10–23 July 1997) application of experimental harvest treatments in Pages and Whiskey Creeks, NC. Sixteen clams were placed in each 1.0-m² quadrat between 5–10 July 1996. Data are means and standard errors (n=4) of counts taken within 1.0-m² quadrats. Results of SNK *post hoc* comparisons are illustrated with letters above bars (a>b at P<0.05). Separate ANOVAs and SNK tests were used to compare numbers of live, dead, and missing clams.

alone also reduced the density of live clams but only at one site, Pages Creek. At Whiskey Creek, the density of live clams after harvesting was similar between oyster-harvested and control plots, indicating that oyster harvesting had little effect on clam survival (Fig. 3). A negative effect of oyster harvesting on clams may be caused both by direct removal of clams as bycatch (Table 2) and enhanced clam mortality through mechanisms analogous to those hypothesized for oysters (see above). Some clams may also have emigrated from the oyster harvesting treatments following harvesting.

Patterns of survival and mortality of hatchery-raised clams transplanted to experimental reefs varied with site and harvest type (Table 5). Fewer live and dead transplanted clams were recovered from reefs at Whiskey Creek than at Pages Creek (Fig. 5). In con-

trast, there were greater numbers of missing transplanted clams at Pages Creek than at Whiskey Creek. Harvest type, specifically clam harvesting, influenced the number of live transplanted clams but had no significant effect on the number of dead or missing transplanted clams. Fewer live, transplanted clams were found in clam-harvested plots than were found in control plots at both sites (Fig. 5). Because few of the transplanted clams were removed from reefs by experimental harvesting, the negative effects of clam harvesting on densities of live, transplanted clams may be explained by increased clam mortality caused by clam harvesting. Overall, the effects of shellfish harvesting appear to be more variable and unpredictable for clams than for oysters. Our results indicate that both types of shellfish harvesting can have negative impacts on clam populations, but that this is a site-specific phenomenon.

Results of this study do not support the hypothesis that harvesting reefs for both clams and oysters has a negative synergistic impact on clam and oyster populations. Clam and oyster harvesting alone had similar negative effects on densities of live oysters, and the joint harvesting of both species on the same reefs did not decrease the density of live oysters any further. Similarly, the negative effects of clam harvesting on the density of live clams, and on survival of hatchery-raised clams were not enhanced when oyster harvesting was applied in combination with clam harvesting. Thus, the combined harvesting of both clams and oysters on intertidal reefs does not cause greater direct or indirect mortality of shellfish populations than that caused by clam or oyster harvesting conducted separately.

This experimental analysis has important implications for the management of intertidal oyster reefs and their associated molluscan fishery resources. First, maintaining high densities of oysters on some intertidal reefs, by preventing both clam and oyster harvesting, may help to preserve future oyster harvests and brood stock. Protecting some reefs from shellfishing will also help preserve the many ecological services that oysters and oyster reefs provide, such as improving water quality through the filtration of suspended particles (Officer et al., 1982; Dame et al., 1984; Newell, 1988) and creating essential recruitment, refuge, and foraging habitat for economically valuable fishes and crabs (Bahr and Lanier, 1981; Zimmerman et al., 1989; Lenihan et al., 1998). Preventing oyster and clam harvesting on some intertidal reefs will also potentially conserve clam populations from both the direct and indirect negative effects of shellfish harvesting, thereby protecting future clam harvests and brood stock. Overall, allowing the harvest of both clams and oysters on some natural and restored oyster reefs is a rational option because the combined effect of both clam and oyster harvesting is no greater than the effect of each harvesting activity conducted alone. Thus, we recommend that both types of harvesting be allowed on some reefs but that other reefs be protected as refuges for shellfish populations and other reef-associated fauna.

In adaptive fishery and habitat management, the results of relatively small-scale, prototype experiments, like the one reported here, are used to design larger-scale comparisons of potential management options. Therefore, we recommend that the results of our experiment be used to design alternative shellfishery management options that can be implemented and monitored on relatively large spatial and temporal scales in North Carolina and other coastal states of North America. Our recommendation that some natural and restored oyster reefs be closed from shellfish harvesting and others opened or restored for the purpose of both clam and oyster harvesting can be used to identify potential management options. Further testing of the generality of our findings on larger spatial and temporal scales is necessary because our study was conducted at only two sites and over a one-year period. Therefore, our results may not apply to areas with different environmental conditions (e.g. different flow and sedimentary regimes, areas of low recruitment) and harvesting intensities (e.g. very low and high levels of harvesting). It is necessary to determine with experiments and simulation models how much oyster reef habitat should be preserved from harvesting to maintain sustainable oyster and clam brood stock populations and habaitat for the successful recruitment and survival of other fishery organisms.

The following steps should now be taken by fishery and habitat managers to improve management of the clam and oyster populations and intertidal oyster reef habitat: 1) identify overall management goals and possible options; 2) derive specific predictions based, at least in part, on the experiment results reported in this study; and 3) design monitoring programs to quantify the effect of each management option. Whenever possible, it is highly recommended that fishermen, fishery managers, and ecologists be included in designing and monitoring large-scale management experiments because collectively they will provide the highest level of rigor and reality.

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Fish assemblages around seven oil platforms in the Santa Barbara Channel area

Milton S. Love

Jennifer E. Caselle

Linda Snook

Marine Science Institute
University of California
Santa Barbara, California 93106
E-mail address: (for M. S. Love): love@lifesci.ucsb.edu

Abstract.–In 1996 we surveyed the fishes living on and around seven offshore oil platforms in the Santa Barbara Channel area. We conducted belt transects at various depths in the midwater and around the bottoms of each platform using the research submersible *Delta*. The bottom depths of these platforms ranged from 49 to 224 m and the midwater beams ranged from 21 to 196 m. We found that there were several distinct differences in the fish assemblages living in the midwater and bottom habitats around all of the platforms. Both midwater and bottom assemblages were dominated by rockfishes. Platform midwaters were dominated by young-of-the-year (YOY) or juveniles up to two years old. Rockfishes larger than about 18 cm total length were rarely seen in the midwater. The fish assemblages around the bottoms of the platforms were dominated by larger individuals, primarily subadults or adults. Density of all fishes was similar between the bottoms and midwater of any given platform. However, the total biomass was much greater on the bottoms, owing to larger fish living there. There was a consistently greater number of species on the bottom than in the midwater of each platform, likely because of a larger variety of habitat types on the bottom. The fish assemblages also differed among platforms. We found significantly higher densities of young-of-the-year rockfishes around platforms north of Pt. Conception compared with those in the Santa Barbara Channel, probably because the more northerly platforms are located in the more productive waters of the California Current.

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Petroleum production has been a part of the southern California economy since the nineteenth century. The earliest drilling took place on land, but by the early twentieth century a large number of piers lined the coast, tapping into offshore oil deposits. Hazel, the first offshore oil platform, was constructed off Summerland in 1958 (Carlisle et al., 1964). At the peak of oil drilling in the early 1980s, there were 30 platforms operating in southern and central California. Currently, there are 19 platforms in operation in the Santa Barbara Channel and off Point Conception (Fig. 1).

Oil platforms provide considerable habitat for marine organisms. The earliest structures were relatively small (23 m long at the surface), newer platforms, however, are over 100 m long (MBC[1]). Sessile invertebrates (primarily mussels, barnacles and anemones) encrust the pilings and well pipes and cover the bottom to form additional habitat.

Oil platforms have a finite economic lifespan and a number of them are becoming uneconomical to operate. In 1996, four platforms were removed from the Santa Barbara Channel, although not without controversy. There is considerable debate regarding the fate of these structures. Some interest groups would like to leave part or all of them in place, claiming protection of fish habitat; others favor complete removal. Understanding the biological communities on the platforms is crucial to making rational decisions regarding the fates of these structures. In addition, research on these platforms could also address questions regarding the role that artificial reefs might play in coastal fish communities. Ultimately, this research will allow us to contrast the fish assemblages on platforms with those of nearby reefs.

Currently, very little is known about the fish fauna around these platforms. One relatively comprehensive SCUBA survey examined fish populations around two shallow inshore platforms, Hazel and Hilda, during Hazel's first three years and Hilda's first year of operation (Carlisle et al., 1964). Additional cursory surveys were conducted around these two platforms in 1970 and 1975; Bascom et al., 1976; Allen and Moore[2]). With the exception of a short-term study of fishes around platform Hidalgo using a remotely operated vehicle (ROV) (Love et al., 1994) and a survey of recreational fishing around Santa Bar-

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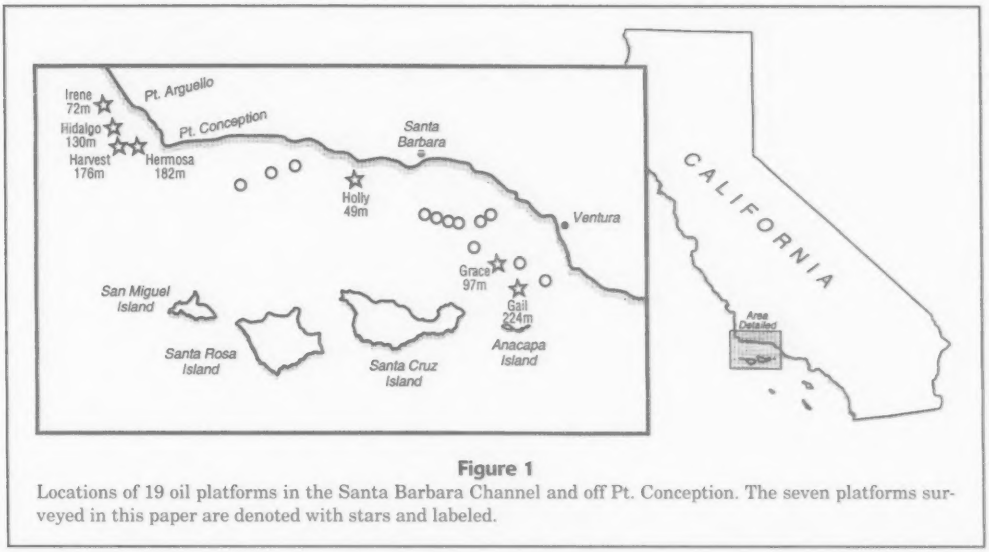

Figure 1
Locations of 19 oil platforms in the Santa Barbara Channel and off Pt. Conception. The seven platforms surveyed in this paper are denoted with stars and labeled.

bara Channel platforms (Love and Westphal, 1990), no other research has been published on the fishes of any California oil platform.

In 1995, we began a survey of the fishes living on and around several platforms in the Santa Barbara Channel area. The surveys were of two types: a scuba-based study in the surface waters (to 30 m) of the platforms and a submersible survey that examined the deeper sections of these structures. However, in 1995, we could not survey any platform bottoms because of inclement weather. This paper discusses the results of the 1996 deep survey.

Materials and methods

Study sites

We surveyed fish assemblages around oil platforms situated in and just northwest of the Santa Barbara Channel. Surveys were conducted around the bottom of six platforms and in the midwater of seven platforms in 1996 (Fig. 1; Table 1). The bottom depth of these platforms ranged from 49 to 224 m. The midwater depths ranged from 21 to 196 m.

The platforms are situated in an area with a complex oceanographic regime. The Santa Barbara Channel is semi-enclosed, faces east-west, and is bordered by the Northern Channel Islands on the south and the mainland on the west. It is embedded within the much larger California-Baja California coastal current regime (Brink and Muench, 1986; Hickey, 1992). Surface waters to the north and west of the Channel are typically cool because the California Current flows equatorward from high latitudes yearround and upwells in the Point Conception and Point Arguello areas during spring and summer. At the same time, the cyclonic circulation pattern in the southern California bight brings warm water flowing poleward along the coast from the east and south of the Santa Barbara Channel. In general, water is cooler and more productive in the area of Points Arguello and Conception than in the Santa Barbara Channel, particularly compared with the more eastern end of the channel.

Surveys

Using the submersible *Delta*, we conducted belt transects around each platform. The submersible maintained a speed of approximately 0.5 knots and stayed approximately 2 m from the structure. Transects were made around the bottom of the platform (from the substrata to approximately 2 m above the substrata) and around each set of cross beams to a minimum depth of about 20 m below the surface. Dives were conducted during daylight hours, between one hour after sunrise and two hours before sunset.

During the transects, researchers made their observations from the central starboard-side viewing port. An externally mounted Hi 8-mm video camera with associated lights filmed the same viewing field as seen by the observers. Observers identified and counted all fishes and verbally recorded those data on the video. All fishes within 2 m of the submarine were counted. Fish lengths were estimated by using

Table 1

Latitude, longitude, bottom and midwater depths, and date of sampling of the seven oil platforms. Platforms listed from northwest to southeast.

Platform	Location	Bottom depth (m)	Midwater depths (m)	Date surveyed
Irene	34°36.62'N, 120°43.77'W	72	29, 50	2 Nov 1996
Hidalgo	34°29.70'N, 120°42.13'W	130	36, 59, 83, 107	28 Oct 1996
Harvest[1]	34°28.15'N, 120°40.85'W	176	38, 61, 84, 113, 141	28 Oct 1996
Hermosa	34°27.33'N, 120°38.78'W	182	63, 84, 106, 131, 156	28 Oct 1996
Holly	34°23.38'N, 120°54.33'W	49	21, 35, 50	29 Oct 1996
Grace	34°10.77'N, 120°28.12'W	97	45, 69, 82	30 Oct 1996
Gail	34°07.50'N, 120°24.02'W	224	71, 95, 115, 141, 166, 196	30 Oct 1996

[1] Midwater survey only.

a pair of lasers mounted on either side of the external video camera. The projected reference spots were 20 cm apart and were visible to the observer. An environmental monitoring system aboard the submarine continuously recorded date and time, depth, and altitude of the vessel above the sea floor. After the dive, these data were overlaid on the original videotape.

Transect videos were reviewed either aboard the research vessel or in the laboratory. For each fish, we recorded 1) its species, to lowest identifiable taxa; 2) its estimated total length to the nearest cm; and 3) the microhabitat it occupied (e.g. pipe, sand, mussel shell mounds, mud). We defined young-of-year fishes (YOYs) from published estimates of size at age. Subadults are defined as juveniles in their second year up to, but not including, maturity.

During the survey at platform Gail, all greenspotted (*Sebastes chlorostictus*) and greenblotched rockfishes (*S. rosenblatti*) were inadvertently identified as greenspotted rockfish. In reviewing the videotape, it was clear that some of the individuals that were recorded as greenspotted rockfish were in fact greenblotched rockfish. In order to correct for this potential misidentification, the total number of both species was adjusted by using the proportion of greenblotched to greenspotted rockfishes (ratio=2.2) observed at platform Gail during the following year's survey (Love, unpub. data). Similar numbers of the two species combined were observed during the two years (1996: n=186,1997: n=209).

Analyses

We estimated length of those transects conducted on the bottom by first determining the submersible speed. This was done by evaluating a ten-second segment for every one minute of transect. The video was manually forwarded frame by frame and the number of 20-cm segments passing the lasers in a ten-second section was counted. The number of 20 cm segments per 10 seconds was divided by 2 to obtain speed in centimeters per second. All subsamples were then averaged to obtain mean transect speed (cm/s). The mean speed was then multiplied by the number of seconds in the transect and divided by 100 to obtain transect length in meters. The length was then multiplied by 2 m (the transect width) to obtain transect area, allowing us to present both densities (fish/m^2) and biomass (kg/m^2). Biomass was estimated for all species by using length-weight relationships derived empirically or obtained from the literature. No biomass estimates were made for species that could not be identified to the family level.

In the midwater, we could not see the lasers pass before fixed points; therefore, we could not directly measure the length of the midwater transects. Without knowledge of the length of the midwater transects, we could not calculate density or biomass per unit area as done on the bottom transects. However, we were able to estimate the length of midwater transects for use in estimating both fish density and biomass. We did this by converting density and biomass on the midwater transects from number and kilogram per minute to number and kilogram per m^2, respectively. This conversion was accomplished by calculating the equation for the regression of density in terms of number per m^2 on density in terms of number per minute for the bottom transects where both values were known (Fig. 2A). The same relationship was calculated for biomass (Fig. 2B). Given the regression equations, density per m^2 and biomass per m^2 could be calculated from number per minute and kilograms per minute. We called these

calculations "estimated density" (number/m^2) and "estimated biomass "(kg/m^2).

This method of estimating transect length and hence fish density and biomass relies on the assumption that the submersible travels at the same speed in both habitats. Although we did not have data on submersible speed, every attempt was made to maintain the submersible at the same speed on all transects during the survey. However, because of debris on the bottom and water currents in the midwater, if there were differences in speed, the submersible was likely to travel slightly faster in the midwater habitats than on the bottoms. In this case, the submersible would cover more area per unit time and the true fish density in the midwater may actually be slightly lower than our estimated density. We consider the potential bias introduced by differences in submersible speed to be minor in relation to the magnitude of the observed differences in fish densities between the midwater and bottom transects (see "Results" section).

We calculated both species richness (number of species) and species diversity. We used the Shannon-Weiner diversity index (H') for all species diversity comparisons (Shannon and Weaver, 1949). We also calculated a percent similarity index (PSI) that quantifies how similar two assemblages are in terms of their species composition (i.e. the relative abundance of those species). The index ranges from 0 (no species shared) to 100% (identical composition and relative abundances). The formula for PSI is

$$PSI = \left\{\sum \min(p_{xi}, p_{yi})\right\} \times 100,$$

where, p_{xi} and p_{yi} are the proportion of the ith species in habitat x and habitat y. PSI was calculated for each pair of platform bottom assemblages.

Results

Bottom versus midwater transects

We found that there were several distinct differences in the fish assemblages living in the midwater and bottom habitats around all of the platforms. We calculated percent similarity indices (PSI) between the bottom and midwater assemblages for each platform. These PSIs ranged from 1% to 34% (mean 13.3%). Although both midwater and bottom assemblages were dominated by rockfishes, platform midwaters were dominated by young-of-the-year (YOY) or slightly older juveniles (<10 cm). Rockfishes larger than about 20 cm were rarely seen in the midwater (Fig. 3). The fish assemblages around the bottoms of the platforms were dominated by subadults or adults (11–20 cm) and occasionally harbored very large individuals (up to 48 cm) (Fig. 3).

Average density per platform of all fishes combined was not significantly different on the bottom versus the midwater transects (bottom mean density

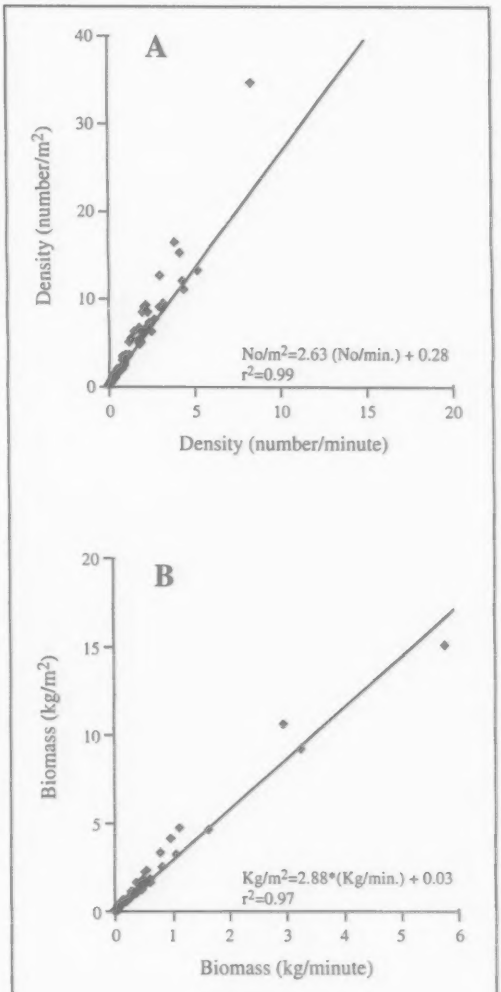

Figure 2

Regressions of (**A**) density in terms of number per minute on density in terms of number per m^2 and (**B**) biomass in terms of kilograms per minute on biomass in terms of kilogram per m^2 for bottom transects on all platforms. The regression equations were used to calculate density and biomass from number and kilogram per minute into number and kilogram per m^2 on the midwater transects. See "Methods" section for explanation of the conversion.

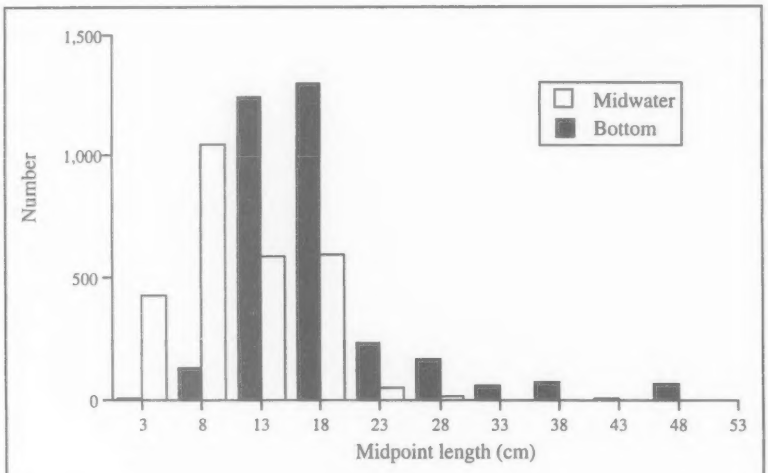

Figure 3
Length-frequency distribution for all rockfish species and all platforms combined. Midpoint length is the midpoint of 5-cm length bins.

(SE)=141.4 fish/100m^2 (49.0), n=6 platforms; midwater mean density (SE)=115.8 fish/100m^2 (32.2), n=7 platforms; t-test, t=0.44, P=0.66). On three platforms, density was higher on the bottom than in midwater and on three other platforms the reverse was true. However, there was a much larger and consistent difference in biomass between bottom and midwater transects. For most families and all platforms, total biomass was higher on bottom than midwater transects (Table 2). Average biomass per platform (SE) for all species combined was 19.06 kg/m^2 (2.5) on the bottom and 6.47 kg/m^2 (2.3) in the midwater (t-test, t=3.75, P=0.003). This consistent difference was due to the lack of adult fishes in the midwater.

Fewer species lived on the midwater structures than on the bottom. Species richness for all rigs combined was 24 in the midwater versus 40 on the bottom. Average species richness per platform was significantly higher on the bottom than in the midwater (bottom mean richness (SE)=14.7 species (1.5); midwater mean richness (SE)=8.2 species (1.4); t-test, t=3.26, P=0.008). Average species diversity (H') across platforms was identical between bottoms and midwaters (bottom mean H' (SE)=1.2 (0.2); midwater mean H' (SE)=1.2 (0.2); t-test, t=0.09, P=0.99). We present the remaining results for bottom and midwater habitats separately.

Bottom habitat

All platforms We identified at least 40 fish species around the platform bottoms (Table 2). Twenty-seven species were rockfishes; they were by far the most speciose group. Rockfishes made up 92.7% of all fishes on the bottom (Table 3) and represented 96.7% of the biomass (Table 2).

Halfbanded, greenspotted, copper, vermilion, widow, and flag rockfishes, and bocaccio were among the most commonly observed rockfishes (Table 3). Our observations indicated that vermilion rockfish, flag rockfish, and bocaccio of all sizes were always closely associated with the platform structure (Fig. 4A). Larger copper and greenspotted rockfishes also were more likely to be very close to the platform. In particular, flag rockfish were most often seen tucked well into the space formed by the bottom of the lowest crossbeam and the bottom (Fig. 4B). Flag and greenspotted rockfishes were almost always seen on or very close to the bottom. Halfbanded rockfish, as well as smaller greenspotted and copper rockfishes, were less bound to the platform and were often seen well away from the structure. Juvenile greenspotted and copper rockfishes were usually nestled within or just above the mussel, shell-covered substrata. Vermilion rockfish, and to a certain extent copper rockfish and bocaccio, would occasionally ascend up platform legs as much as 5 m.

Flag rockfish, as well as larger bocaccio and vermilion rockfish, often were solitary or found in small groups. The exception occurred at platform Gail, where one school of bocaccio comprised at least 100 individuals. Smaller adult or subadult vermilion and copper rockfishes tended to aggregate, often in mixed groups containing 50 or more individuals. The few

Table 2

Biomasses of fishes observed, by species, around all platforms in October–November of 1996. Biomasses are given for bottoms of platforms and midwater, with percent of totals for those parts of the platforms. Biomass is kilograms/m^2. Family totals are given in boldface. YOY means "young-of-year."

			Bottom		Midwater	
Family	Common name	Scientific name	Biomass	% Total	Biomass	% Total
Scorpaenidae	Rockfishes		**96.83**	**84.66**	**38.81**	**85.90**
	Kelp rockfish	*Sebastes atrovirens*	0	0	0.34	0.75
	Brown rockfish	*S. auriculatus*	0.82	0.71	0	0
	Gopher rockfish	*S. carnatus*	0.01	<0.1	0.23	0.51
	Copper rockfish	*S. caurinus*	12.12	10.59	0.47	1.04
	Greenspotted rockfish	*S. chlorostictus*	8.80	10.94	0.56	1.23
	Starry rockfish	*S. constellatus*	0.02	<0.1	0	0
	Darkblotched rockfish	*S. crameri*	0.07	<0.1	0	0
	Calico rockfish	*S. dalli*	1.40	1.22	0.08	0.18
	Greenstriped rockfish	*S. elongatus*	0.32	0.28	0	0
	Swordspine rockfish	*S. ensifer*	0.03	<0.1	0.08	0.17
	Widow rockfish	*S. entomelas*	1.86	1.62	24.08	53.15
	Yellowtail rockfish	*S. flavidus*	0.08	<0.1	0	0
	Chilipepper	*S. goodei*	0	0	0.82	1.82
	Squarespot rockfish	*S. hopkinsi*	0.46	0.40	0.51	1.12
	Vermilion rockfish	*S. miniatus*	20.84	18.22	0	0
	Blue rockfish	*S. mystinus*	0.74	0.65	0.29	0.64
	Bocaccio rockfish	*S. paucispinis*	14.35	12.55	3.68	8.12
	Canary rockfish	*S. pinniger*	1.18	1.03	0	0
	Rosy rockfish	*S. rosaceus*	0.46	0.40	0.07	0.15
	Greenblotched rockfish	*S. rosenblatti*	3.89	0.15	0	0
	Yelloweye rockfish	*S. ruberrimus*	0.06	<0.1	0	0
	Flag rockfish	*S. rubrivinctus*	1.44	1.26	0.55	1.21
	Bank rockfish	*S. rufus*	0.29	0.25	0	0
	Halfbanded rockfish	*S. semicinctus*	26.21	22.91	0.04	<0.1
	Olive rockfish	*S. serranoides*	0.03	<0.1	0	0
	Treefish	*S. serriceps*	0.19	0.17	0.04	<0.1
	Pygmy rockfish	*S. wilsoni*	0.04	<0.1	0	0
	Sharpchin rockfish	*S. zacentrus*	0.10	<0.1	0.04	<0.1
	Shortspine thornyhead	*Sebastolobus alascanus*	<0.1	<0.1	0	0
	Sebastomus group[1]		0.31	0.27	0.37	0.82
	Rockfish YOY	*Sebastes* spp.	0.72	0.63	6.56	14.47
Hexagrammidae	Greenlings		**12.88**	**11.25**	**2.72**	**6.01**
	Kelp greenling	*Hexagrammos decagrammus*	0	0	<0.1	<0.1
	Lingcod	*Ophiodon elongatus*	12.35	10.80	0	0
	Painted greenling	*Oxylebius pictus*	0.51	0.44	2.72	6.01
	Shortspine combfish	*Zaniolepis frenata*	0.01	<0.1	0	0
	Combfish sp.	*Zaniolepis* sp.	0.01	<0.1	0	0
Pomacentridae	Damselfishes		**0**	**0**	**0.54**	**1.20**
	Blacksmith	*Chromis punctipinnis*	0	0	0.54	1.20
Embiotocidae	Seaperches		**4.57**	**3.99**	**3.02**	**6.65**
	Pile perch	*Damalichthys vacca*	3.71	3.24	1.18	2.60
	Sharpnose surfperch	*Phanerodon atripes*	0.49	0.43	1.84	4.05
	Unident. sea perches		0.20	0.17	0	0
	Pink surfperch	*Zalembius rosaceus*	0.17	0.15	0	0
Gadidae	Cods		**0**	**0**	**0.20**	**0.44**
	Pacific hake	*Merluccius productus*	0	0	0.20	0.44

continued

Table 2 (continued)

Family	Common name	Scientific name	Bottom		Midwater	
			Biomass	% Total	Biomass	% Total
Cottidae	Sculpins		**0**	**0**	**0.03**	**0.07**
	Unidentifed sculpin		0	0	0.03	0.07
Bathymasteridae	Ronquils		**0.03**	**0.03**	**0**	**0**
	Unidentified ronquil		0.03	0.03	0	0
Agonidae	Poachers		**0.01**	**0.01**	**0**	**0**
	Unidentified poacher		0.01	0.01	0	0
Flatfish	Flatfish		**0.06**	**0.06**	**0**	**0**
	Sanddabs	*Citharichthys* sp.	0.01	0.01	0	0
	Unident. flatfish		0.05	0.05	0	0

[1] *Sebastomus* group may include greenblotched, greenspotted, pinkrose, rosethorn, rosy, starry, or swordspine rockfishes.

canary rockfish we noted tended to associate with vermilion rockfish. Halfbanded rockfish were almost always seen in schools, sometimes containing hundreds of individuals (Fig. 4C).

In the greenling family, Hexagrammidae, both lingcod and painted greenling, were common; together they represented about 5.3% of all fishes seen. Larger lingcod were solitary and tended to remain near the bottom of the platform (Fig. 4D). They were usually seen sitting motionless on the bottom or slowly swimming just above it. Juvenile lingcod rarely came within a meter of the platform, they were usually seen lying among the mussel shells away from the structure (Fig. 4E). Painted greenling sat on the crossbeams, along the pilings and on the mussel shells, always found as solitary individuals.

Among platform comparisons The bottom fish assemblages around each platform were all different (Tables 4 and 5). Pairwise percent similarity indices (PSI) for each combination of platforms ranged from 0% (platforms Gail and Holly) to 70.1% (platforms Grace and Hidalgo) (Table 4). The average percent similarity was 20.0%. Despite a low average similarity value, rockfishes, as measured by number, density and biomass, dominated the bottom assemblages around all of the platforms (Table 5). Lingcod were the only nonrockfish species among the top four most common species at any platform.

Around platform Irene, subadult and adult copper and vermilion rockfishes were most abundant. Irene also was unique among the platforms in having large numbers of juvenile lingcod. Halfbanded rockfish, painted greenling, and pile perch were also commonly seen. Halfbanded and greenspotted rockfish were most common at platform Hidalgo, along with flag rockfish, lingcod, bocaccio, and vermilion rockfish. Similar to that around Hidalgo (PSI=60%), the bottom fish assemblage around platform Hermosa was characterized by greenspotted and halfbanded rockfish, with lesser numbers of flag rockfish and lingcod (Table 4). Vermilion, calico, widow, copper, and squarespot rockfishes were most often seen at Holly, along with lesser numbers of halfbanded rockfish, pile perch, rosy rockfish, and painted greenling. Very large schools of halfbanded rockfish were observed at Grace, along with some flag, greenspotted, and vermilion rockfishes. The dominance of halfbanded rockfish at Hidalgo and Grace resulted in the highest PSI among platform pairs (70.1%). Members of the rockfish subgenus *Sebastomus*, primarily greenblotched and greenspotted rockfishes and bocaccio were most abundant at platform Gail. Gail had by far the highest number and density of bocaccio of any of the platforms.

We observed between 8 and 21 species around the bottom of the platforms (Fig. 5A). We found no significant relationship between species number or diversity (H') and platform bottom depth (linear regression: species richness vs. depth, r^2=0.58, P=0.07, diversity (H') vs. depth, r^2=0.19, P=0.37, Fig. 5A). Although neither relationship was significant, there was a tendency for platforms in shallower water to have both higher species richness and species diversity. Location of the platforms within the Santa Barbara Channel and Santa Maria Basin also did not explain the differences among platforms. There was no correlation between northwest to southeast orientation and either species richness or diversity (Spearman rank correlation: species richness vs. orientation, r_s=–0.26, P=0.6, diversity (H') vs. orientation, r_s=–0.6, P=0.2). In fact, the two most

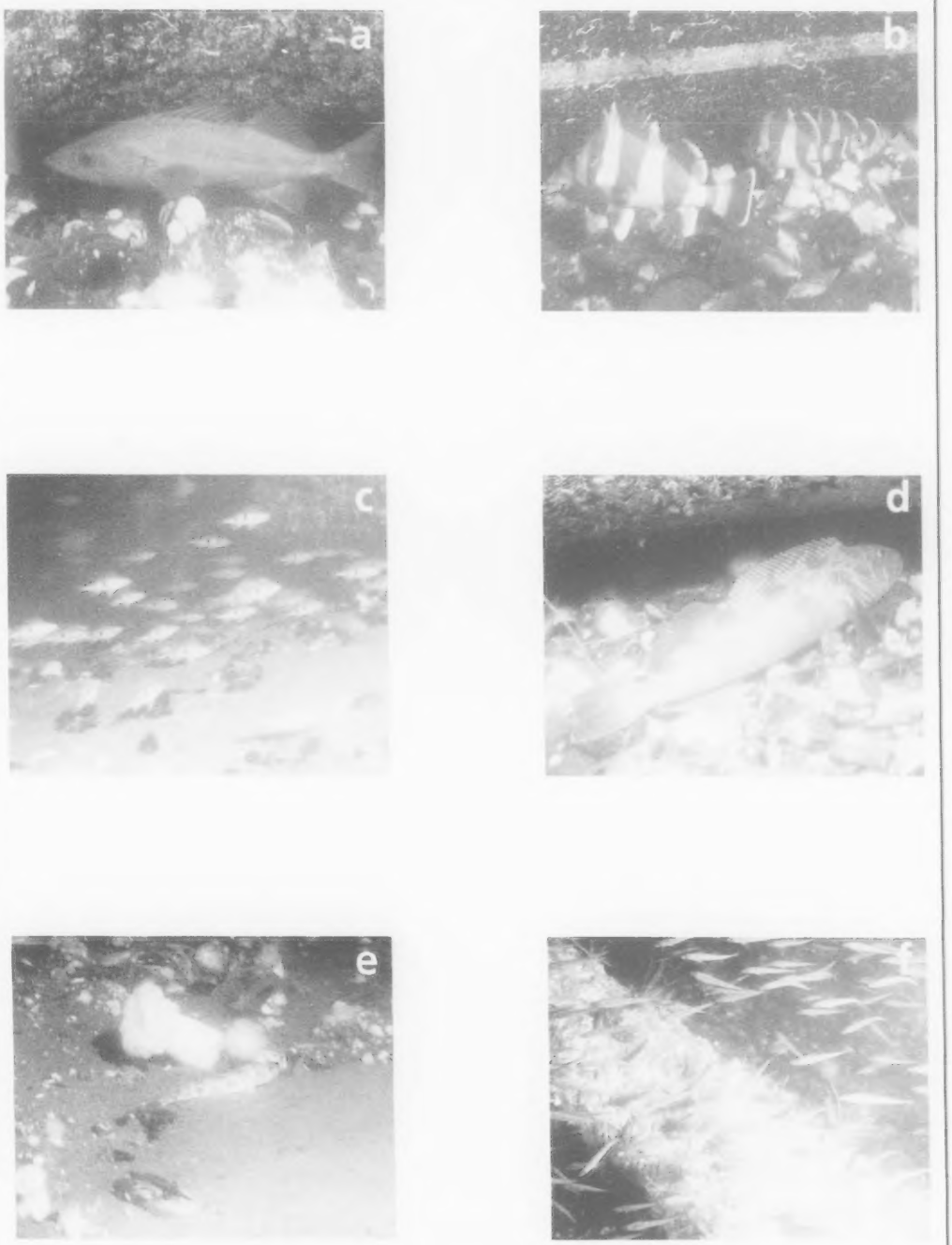

Figure 4

Fishes typical of offshore oil platforms in the Santa Barbara Channel and Santa Maria Basin: (**A**) bocaccio, *Sebastes paucispinis*, (**B**) flag rockfish, *S. rubrivinctus*, (**C**) halfbanded rockfish, *S. semicinctus*, (**D**) adult lingcod, *Ophiodon elongatus*, (**E**) juvenile lingcod, A–E all on bottom transects, and (**F**) young-of-the-year rockfish, *Sebastes* spp,. on midwater crossbeam.

Table 3

Numbers of fishes observed, by species, around all platforms in October–November of 1996. Numbers are given for bottoms of platforms and midwater, with percent of totals for those parts of the platforms. Family totals are given in boldface. YOY means "young-of-year."

			Bottom		Midwater	
Family	Common name	Scientific name	Biomass	% Total	Biomass	% Total
Scorpaenidae	Rockfishes		**4212**	**92.7**	**2753**	**91.4**
	Kelp rockfish	*Sebastes atrovirens*	0	0	1	<0.1
	Brown rockfish	*S. auriculatus*	7	0.2	0	0
	Gopher rockfish	*S. carnatus*	2	<0.1	4	0.1
	Copper rockfish	*S. caurinus*	347	7.6	11	0.4
	Greenspotted rockfish	*S. chlorostictus*	365	8.0	18	0.6
	Starry rockfish	*S. constellatus*	1	<0.1	0	0
	Darkblotched rockfish	*S. crameri*	1	<0.1	0	0
	Calico rockfish	*S. dalli*	68	1.5	2	<0.1
	Greenstriped rockfish	*S. elongatus*	12	0.3	0	0
	Swordspine rockfish	*S. ensifer*	2	<0.1	2	<0.1
	Widow rockfish	*S. entomelas*	115	2.5	1054	35.0
	Yellowtail rockfish	*S. flavidus*	1	<0.1	0	0
	Chilipepper	*S. goodei*	0	0.00	68	2.3
	Squarespot rockfish	*S. hopkinsi*	47	1.0	22	0.7
	Vermilion rockfish	*S. miniatus*	307	6.8	0	0
	Blue rockfish	*S. mystinus*	7	0.2	6	0.2
	Bocaccio rockfish	*S. paucispinis*	85	1.9	264	8.8
	Canary rockfish	*S. pinniger*	10	0.2	0	0
	Rosy rockfish	*S. rosaceus*	31	0.7	1	<0.1
	Greenblotched rockfish	*S. rosenblatti*	129	2.8	0	0
	Yelloweye rockfish	*S. ruberrimus*	2	<0.1	0	0
	Flag rockfish	*S. rubrivinctus*	113	2.5	15	0.5
	Bank rockfish	*S. rufus*	2	<0.1	0	0
	Halfbanded rockfish	*S. semicinctus*	2491	54.8	1	<0.1
	Olive rockfish	*S. serranoides*	1	<0.1	0	0
	Treefish	*S. serriceps*	5	0.1	1	<0.1
	Pygmy rockfish	*S. wilsoni*	4	<0.1	0	0
	Sharpchin rockfish	*S. zacentrus*	11	0.2	1	<0.1
	Shortspine thornyhead	*Sebastolobus alascanus*	1	<0.1	0	0
	Sebastomus group[1]		19	0.4	13	0.4
	Rockfish YOY	*Sebastes* spp.	26	0.6	1269	42.1
Hexagrammidae	Greenlings		**244**	**5.4**	**187**	**6.2**
	Kelp greenling	*Hexagrammos decagrammus*	0	0	1	<0.1
	Lingcod	*Ophiodon elongatus*	193	4.3	0	0
	Painted greenling	*Oxylebius pictus*	46	1.0	186	6.2
	Shortspine combfish	*Zaniolepis frenata*	2	<0.1	0	0
	Combfish sp.	*Zaniolepis* sp.	3	<0.1	0	0
Pomacentridae	Damselfishes		**0**	**0**	**12**	**0.4**
	Blacksmith	*Chromis punctipinnis*	0	0	12	0.4
Embiotocidae	Seaperches		**65**	**1.4**	**20**	**0.7**
	Pile perch	*Damalichthys vacca*	46	1.0	6	0.2
	Sharpnose surfperch	*Phanerodon atripes*	9	0.2	14	0.5
	Unident. sea perches		1	<0.1	0	0
	Pink surfperch	*Zalembius rosaceus*	9	0.2	0	0
Gadidae	Cods		**2**	**<0.1**	**18**	**0.6**
	Pacific hake	*Merluccius productus*	2	<0.1	18	0.6

continued

Table 3 (continued)

Family	Common name	Scientific name	Bottom		Midwater	
			Biomass	% Total	Biomass	% Total
Cottidae	Sculpins		**0**	**0**	**1**	**<0.1**
	Unidentifed sculpin		0	0	1	<0.1
Bathymasteridae	Ronquils		**2**	**<0.1**	**0**	**0**
	Unidentified ronquil		2	<0.1	0	0
Agonidae	Poachers		**2**	**<0.1**	**0**	**0**
	Unidentified poacher		2	<0.1	0	0
Flatfish	Flatfish		**13**	**0.3**	**0**	**0**
	Sanddabs	*Citharichthys* sp.	1	<0.1	0	0
	Unident. flatfish		12	0.3	0	0

[1] *Sebastomus* group may include greenblotched, greenspotted, pinkrose, rosethorn, rosy, starry, or swordspine rockfishes.

similar assemblages were on platforms near the geographic extremes (Hidalgo and Grace).

Density and biomass of all species combined also varied among rigs but in a pattern different from species richness and diversity (Fig. 5B). However, similar to richness and diversity, density and biomass differences could not be explained by bottom depth or by geography (linear regression: density vs. depth, r^2=0.22, P=0.35, biomass vs. depth, r^2=0.06, P=0.64; Spearman rank correlation: density vs. orientation, r_s=-0.31, P=0.54, biomass vs. orientation, r_s=-0.37, P=0.46).

Although bottom depth did not explain the patterns of abundance of all species combined, the abundance patterns of individual species did relate more strongly to bottom depth. Among the more commonly observed species, eight showed depth-related patterns of abundance (Fig. 6). Copper and vermilion rockfishes, lingcod, and painted greenling were most dense around the bottoms of some of the shallower platforms (especially platform Irene). Halfbanded and flag rockfishes were most dense on the bottoms of the middepth structures and bocaccio and greenspotted rockfish were most common at the bottom of the deeper platforms.

Table 4

Percent similarity indices for each pair of platforms for the bottom only in 1996. No bottom surveys were done on platform Harvest.

Platform	Gail	Grace	Holly	Hermosa	Hidalgo
Grace	2.3				
Holly	0	8.5			
Hermosa	24.7	34.5	8.5		
Hidalgo	17.9	70.1	11.8	60.0	
Irene	1.4	4.7	41.5	3.8	10.0

Midwater habitat

All platforms Rockfishes also dominated the midwater portions of the platforms, but were primarily YOYs and slightly older juveniles. Rockfishes represented 91.4% of the individuals (Table 3) and 85.9% of the biomass (Table 2) in the midwater. Although it was difficult to identify many of the smaller individuals, widow rockfish were by far the most common species, representing 35.0% of all fishes seen. It is likely that many of the small, unidentifiable YOYs also were widow rockfish. YOY bocaccio also were fairly abundant around some of the platforms and occasionally schooled with widow rockfish. Both species formed relatively tight, polarized schools, loosely associated with the pilings and crossbeams (Fig. 4F). When disturbed, the schools immediately swam inward underneath the platform structure. We also saw small numbers of what we tentatively identified as YOYs of the complex of kelp, copper, gopher and black-and-yellow rockfishes (*S. chrysomelas*). These were found in smaller, much less coherent aggregations and were more likely to move in closer to the substrata when disturbed.

Painted greenling, primarily small individuals, were the most commonly seen nonrockfish species. We often saw solitary individuals resting on the crossbeams. Other species occasionally seen near or

Table 5

Number, densities, and biomasses of fishes observed around the bottoms of six oil platforms off central and southern California. Platforms are listed geographically, from northwest to southeast. Species are ranked by number observed. YOY means "young-of-year." We computed minimum number of species by assuming that each unidentified taxa (flatfish, poacher, ronquil and seaperch) represented one species.

Platform	Species	Number	Density (fish/100m^2)	Biomass (kg/m^2)
Irene	Copper rockfish	297	55.99	10.01
	Vermilion rockfish	198	37.33	11.81
	Lingcod	152	28.65	1.23
	Halfbanded rockfish	25	4.71	0.16
	Painted greenling	20	3.77	0.24
	Pile perch	20	3.77	0.83
	Rosy rockfish	4	0.75	0.02
	Sebastomus group[1]	2	0.38	0.01
	Brown rockfish	2	0.38	0.05
	Bocaccio	1	0.19	0.24
	Flag rockfish	1	0.19	0.01
	Gopher rockfish	1	0.19	0.00
	Rockfish YOY	1	0.19	0.01
	Widow rockfish	1	0.19	0.02
	Yellowtail rockfish	1	0.19	0.08
	Total	**726**	**136.86**	**24.73**
	Minimum number of species	13		
Hidalgo	Halfbanded rockfish	552	94.62	9.35
	Greenspotted rockfish	109	18.68	3.48
	Flag rockfish	58	9.94	1.00
	Lingcod	29	4.97	6.52
	Bocaccio	17	2.91	2.10
	Vermilion rockfish	13	2.23	2.83
	Rosy rockfish	10	1.71	0.27
	Sharpchin rockfish	10	1.71	0.09
	Canary rockfish	7	1.20	1.03
	Greenstriped rockfish	7	1.20	0.11
	Painted greenling	5	0.86	0.07
	Pygmy rockfish	4	0.69	0.04
	Widow rockfish	4	0.69	0.64
	Squarespot rockfish	3	0.51	0.03
	Rockfish YOY	2	0.34	0.06
	Shortspine combfish	2	0.34	0.01
	Yelloweye rockfish	2	0.34	0.06
	Sebastomus group [1]	1	0.17	0.01
	Bank rockfish	1	0.17	0.02
	Unidentified poacher	1	0.17	0.01
	Total	**837**	**143.47**	**27.73**
	Minimum number of species	18		
Hermosa	Greenspotted rockfish	179	25.72	3.24
	Halfbanded rockfish	98	14.08	0.71
	Flag rockfish	16	2.30	0.20
	Lingcod	7	1.01	2.60
	Rockfish YOY	5	0.72	0.10
	Copper rockfish	4	0.57	0.03
	Pacific hake	2	0.29	0.00
	Sebastomus group[1]	1	0.14	0.04
	Greenblotched rockfish	1	0.14	0.15

continued

Table 5 (continued)

Platform	Species	Number	Density (fish/100m^2)	Biomass (kg/m^2)
Hermosa continued	Greenstriped rockfish	1	0.14	0.01
	Unidentified poacher	1	0.14	0.00
	Sharpchin rockfish	1	0.14	0.01
	Starry rockfish	1	0.14	0.02
	Widow rockfish	1	0.14	0.02
	Total	**318**	**45.70**	**7.13**
	Minimum number of species	12		
Holly	Vermilion rockfish	87	21.98	5.87
	Calico rockfish	68	17.18	1.40
	Widow rockfish	47	11.88	1.14
	Copper rockfish	45	11.37	2.05
	Squarespot rockfish	43	10.87	0.41
	Halfbanded rockfish	29	7.33	0.12
	Pile perch	26	6.57	2.88
	Rosy rockfish	16	4.04	0.11
	Painted greenling	15	3.79	0.17
	Sharpnose surfperch	9	2.27	0.49
	Blue rockfish	7	1.77	0.74
	Pink surfperch	7	1.77	0.15
	Unident. flatfish	6	1.52	0.02
	Brown rockfish	5	1.26	0.77
	Sebastomus group [1]	4	1.01	0.08
	Canary rockfish	3	0.76	0.15
	Rockfish YOY	3	0.76	0.15
	Treefish	2	0.51	0.10
	Ronquils	1	0.25	0.00
	Unident. sea perches	1	0.25	0.20
	Combfish sp.	1	0.25	0.00
	Gopher rockfish	1	0.25	0.01
	Olive rockfish	1	0.25	0.03
	Shortspine thornyhead	1	0.25	0.00
	Unidentified fish	1	0.25	•
	Total	**429**	**108.40**	**17.04**
	Minimum number of species	21		
Grace	Halfbanded rockfish	1787	351.16	15.87
	Flag rockfish	30	5.90	0.18
	Greenspotted rockfish	18	3.54	0.38
	Vermilion rockfish	9	1.77	0.33
	Rockfish YOY	7	1.38	0.05
	Unident. flatfish	6	1.18	0.03
	Painted greenling	6	1.18	0.03
	Widow rockfish	5	0.98	0.05
	Treefish	3	0.59	0.09
	Combfish sp.	2	0.39	0.01
	Lingcod	2	0.39	0.03
	Pink surfperch	2	0.39	0.01
	Ronquils	1	0.20	0.03
	Copper rockfish	1	0.20	0.03
	Greenblotched rockfish	1	0.20	0.02
	Rosy rockfish	1	0.20	0.06
	Sanddabs	1	0.20	0.01
	Squarespot rockfish	1	0.20	0.01

continued

Table 5 (continued)

Platform	Species	Number	Density (fish/100m^2)	Biomass (kg/m^2)
Grace continued	Unidentified fish	1	0.20	•
	Total	**1884**	**370.22**	**17.21**
	Minimum number of species	16		
Gail	Greenblotched rockfish	127	19.8	3.71
	Bocaccio	67	10.46	12.01
	Greenspotted rockfish	59	9.2	1.70
	Sebastomus group [†]	10	1.9	0.18
	Rockfish YOY	5	0.78	0.35
	Greenstriped rockfish	4	0.62	0.20
	Lingcod	3	0.47	1.96
	Flag rockfish	2	0.31	0.03
	Swordspine rockfish	2	0.31	0.03
	Bank rockfish	1	0.16	0.27
	Darkblotched rockfish	1	0.16	0.07
	Total	**281**	**44.17**	**20.51**
	Minimum number of species	9		

[†] *Sebastomus* group may include greenblotched, greenspotted, pinkrose, rosethorn, rosy, starry, or swordspine rockfishes.

on midwater structure included juvenile greenspotted and flag rockfishes, as well as sharpnose seaperch, pile perch, and blacksmith.

Among platform comparisons The midwater assemblages also differed among rigs, although the variability was less than among the bottom assemblages. Species richness ranged from 6 to 11 species per platform (Fig. 7A). Species diversity also showed less variability among platform midwaters than platform bottoms (midwater H' range: 0.7 to 1.8, Fig. 7A).

The midwater around platform Irene was dominated by widow rockfishes (primarily YOYs, but also one-year-old fishes), unidentified YOY rockfishes (probably primarily widow rockfish) and YOY bocaccio. Almost no other fishes were noted (Table 6). The species composition at platforms Hidalgo and Harvest was similar to that at platfrom Irene, although painted greenling were also occasionally seen. Far fewer fishes were noted at platform Hermosa, although the species composition was similar, with the addition of small numbers of Pacific hake. Fewer fishes were seen at platform Holly. Here, YOY rockfish (probably widow rockfish), painted greenling, sharpnose seaperch and squarespot rockfish were the most common species. Smaller numbers of juvenile widow rockfish, YOY rockfish, and juvenile chilipepper characterized platform Grace. We saw fewest fishes in the midwaters around platform Gail where YOY rockfish (again probably widow rockfish) were the most common.

In general, the platforms at the western end of the Santa Barbara Channel harbored a higher density of fishes in the midwater than did those towards the east (Fig. 7B). There was a significant relationship between density and northwest-southeast rank (Spearmans r_s=0.89, P=0.006). This pattern was due to higher density of YOY rockfishes, especially widow rockfish and bocaccio, at platforms Irene and Hidalgo. YOY rockfishes were abundant only at Irene and Hidalgo, they were much less common at the platforms farther east. There was not a significant relationship between biomass and northwest-southeast rank (Spearmans r_s=0.64, P=0.11).

Length-frequency comparisons

Relatively few species were abundant in both the midwater and bottom assemblages. For those species that were found in both environments, such as copper, flag, and greenspotted rockfishes, there was a tendency for juveniles to be found in the midwater and older individuals on the bottom. Bocaccio were the extreme example, with smaller juveniles occurring only in midwater and larger individuals only on the bottom (Fig. 8). The painted greenling was one of the few species that occurred in virtually all size classes in both the midwater and on the bottom (Fig. 8).

There were considerable differences in the size frequencies of the major species around the platforms (Fig. 8). Some species, such as copper rockfish and vermilion rockfish, were found primarily as juveniles and subadults. At the extreme, we did not identify any mature widow rockfish. Numerous other species (i.e. painted greenling, bocaccio, greenspotted rockfish,

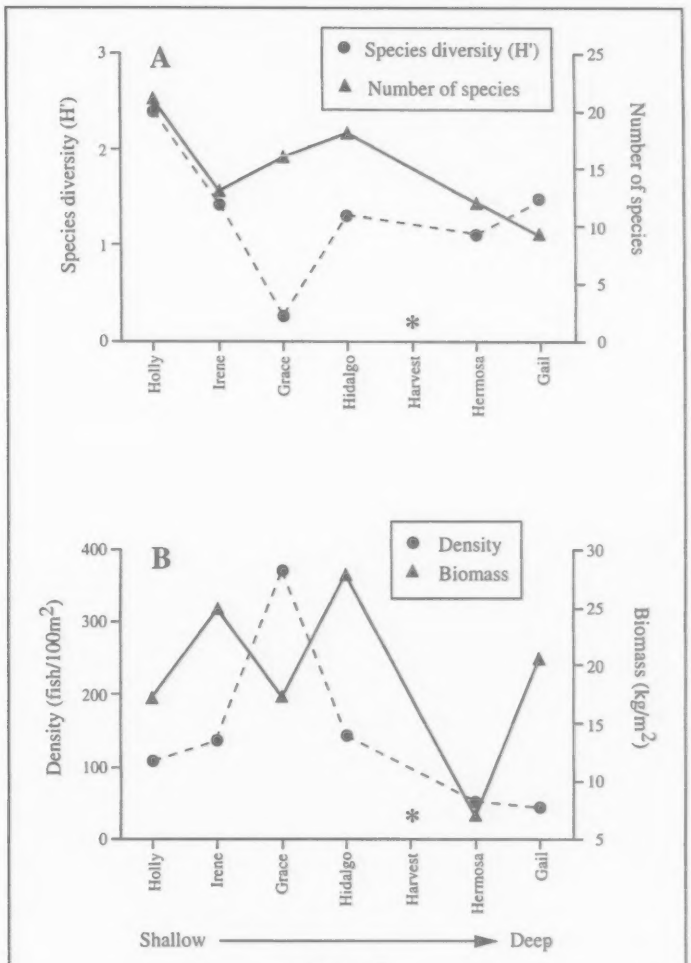

Figure 5

(**A**) Species richness (number of species) and species diversity (Shannon-Weaver diversity index, H') and (**B**) density (fish/100 m^2) and biomass (kilograms/m^2) on the bottoms of seven platforms in the Santa Barbara Channel area. Platforms are ordered by bottom depth from shallow (Holly) to deep (Gail). * = no bottom transects done.

flag rockfish, and halfbanded rockfish) were found over a wide size range, encompassing most life stages. Although a wide size range of lingcod was observed, it is noteworthy that most of the small fish were found around platform Irene; relatively few of these young individuals inhabited the other platforms.

Discussion

Although we found large variability in many of the attributes of the fish assemblages living around these seven oil platforms, several consistent patterns were evident. Around all of these structures, the midwater fish assemblage was quite different from that inhabiting the platform bottoms. Juvenile rockfishes were by far the dominant group occupying the midwater. Although the density of all species combined was similar between the bottom and midwater of any given platform, the biomass was much greater on the bottom, owing to larger fish living around the bottom. In addition, there was a consistently greater number of species on the bottom than in the midwater around each platform. The bottom of the

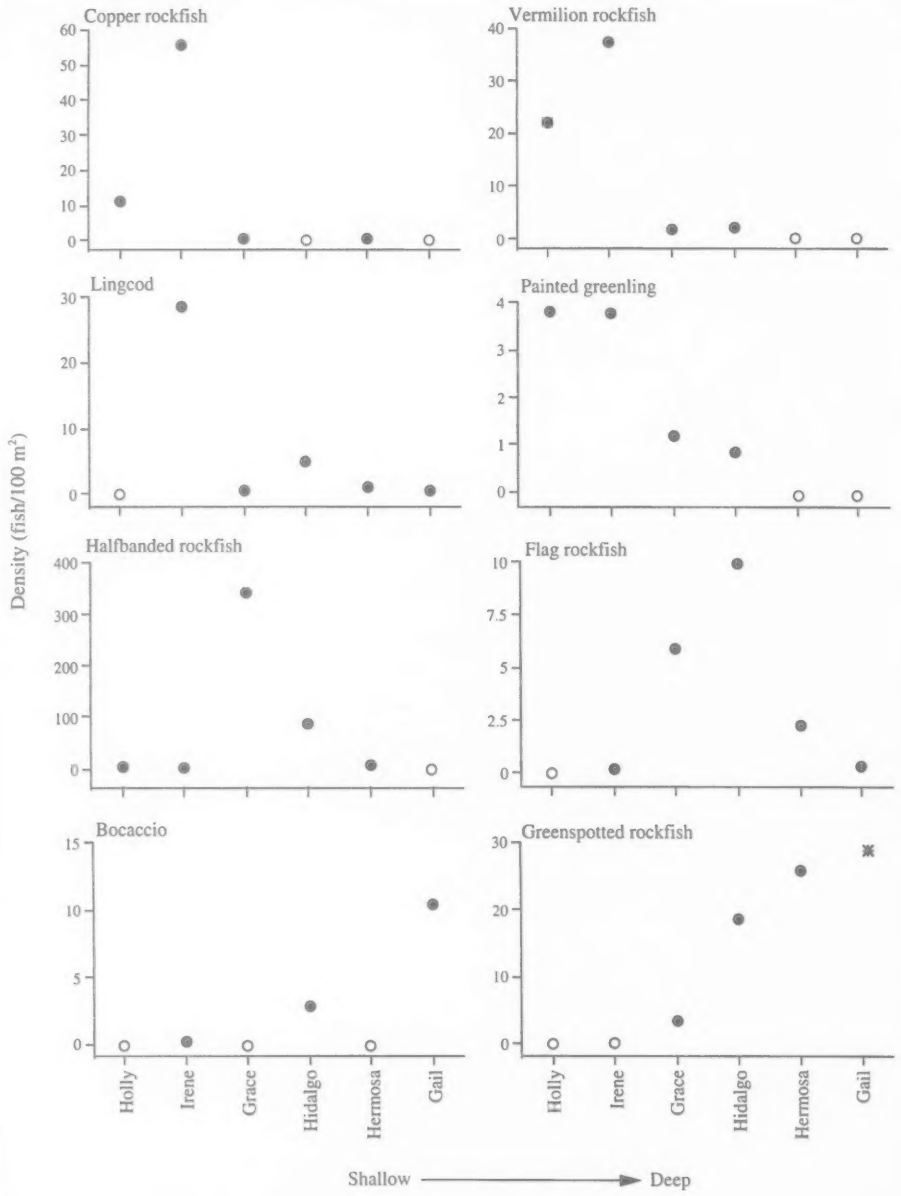

Figure 6

Densities (fish/100m²) of eight common species on the bottoms of seven platforms in the Santa Barbara Channel area. Platforms are ordered by bottom depth from shallow (Holly) to deep (Gail). Data for greenspotted rockfish around platform Gail (noted by *), may include observation of greenblotched rockfish. Empty symbols represent zero values.

platforms provided a larger variety of habitat types than did the midwater. Bottoms are often largely composed of shell mounds that have fallen from the upper parts of the platforms. These mounds, in com-

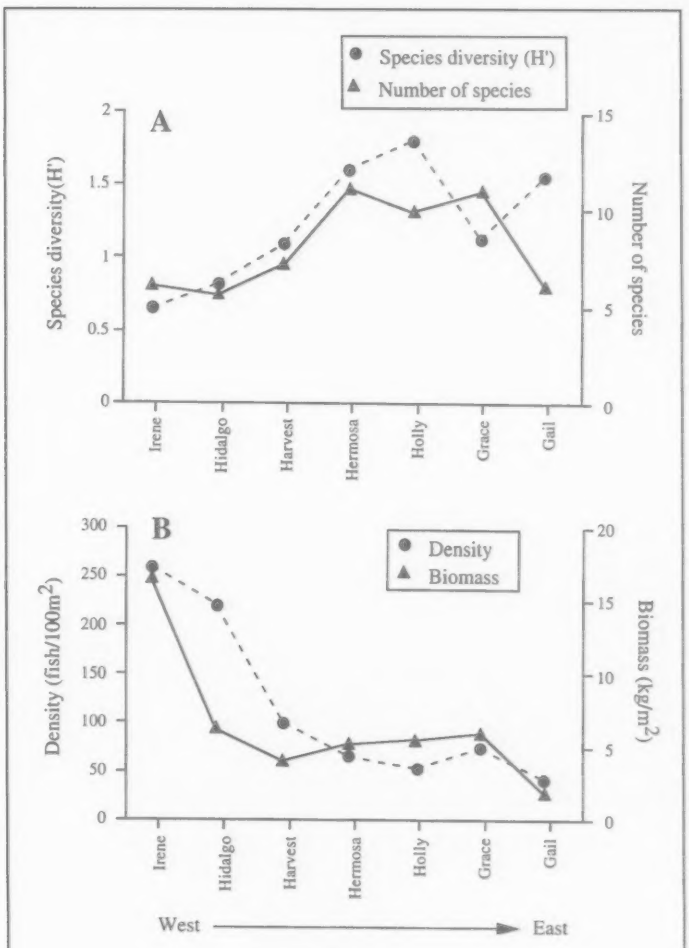

Figure 7

(**A**) Species richness (number of species) and species diversity (Shannon-Weaver diversity index, H') and (**B**) density (fish/100 m^2) and biomass (kilograms/m^2) on the midwater transects of seven platforms in the Santa Barbara Channel area. Platforms are ordered by geography from northwest (Irene) to southeast (Gail).

bination with the wells, crossbeams, and pilings provide a greater degree of habitat complexity and thus, may allow a greater number of species to coexist.

Platforms north of Pt. Conception in the Santa Maria Basin contain far more YOY rockfishes than those in the Santa Barbara Channel to the south. This geographic difference is almost certainly due to the difference in water masses of the two areas. Platforms north of Pt. Conception are more exposed to the California Current; those south of the Point are more influenced by Southern California Bight water (Brink and Muench, 1986; Hickey, 1992). There is considerable evidence that, within much of the Southern California Bight, juvenile rockfish recruitment has been poor for a number of years (Stephens et al., 1984, 1994; Love et al., 1998), probably due to decadal-long changes in oceanographic conditions. Since the late 1970s, waters off Southern California have warmed significantly and upwelling has declined. This situation has led to reduced zooplankton production (Roemmich and McGowan, 1995) and a reduction in the survival of many marine fish species in early life stages (Holbrook and Schmitt, 1996). The present regime is probably part of a long-term

Table 6

Number, densities, and biomasses of fishes observed on the midwater transects of seven oil platforms off central and southern California. Platforms are listed geographically, from northwest to southeast. YOY means "young-of-year." We computed minimum number of species by assuming that each unidentified taxa (flatfish, poacher, ronquil, and seaperch) represented one species. Both density and biomass on midwater transects are estimates calculated from transect minutes (see text for explanation of the conversion).

Platform	Species	Number	Estimated density (fish/100m^2)	Estimated biomass (kg/m^2)
Irene	Widow rockfish	447	127.25	12.47
	Rockfish YOY	271	78.22	1.36
	Bocaccio	162	46.56	2.73
	Blue rockfish	2	0.85	0.07
	Copper rockfish	2	0.85	0.11
	Pile perch	2	1.14	0.19
	Painted greenling	1	0.57	0.04
	Total	**887**	**255.44**	**16.97**
	Minimum number of species	6		
Hidalgo	Rockfish YOY	647	137.88	1.80
	Widow rockfish	286	50.57	3.62
	Bocaccio	78	19.70	0.29
	Painted greenling	29	8.60	0.28
	Greenspotted rockfish	2	0.71	0.04
	Unident. sculpin	1	0.53	0.03
	Flag rockfish	1	0.46	0.04
	Total	**1044**	**218.46**	**6.10**
	Minimum number of species	6		
Harvest	Widow rockfish	171	39.45	2.51
	Rockfish YOY	102	24.80	0.42
	Bocaccio	43	11.53	0.18
	Painted greenling	36	11.78	0.51
	Unidentified fish	17	5.09	•
	Blacksmith	8	2.27	0.05
	Greenspotted rockfish	5	1.80	0.11
	Calico rockfish	1	0.55	0.05
	Flag rockfish	1	0.45	0.03
	Total	**384**	**97.72**	**3.87**
	Minimum number of species	7		
Hermosa	Painted greenling	77	21.24	1.06
	Rockfish YOY	63	17.70	1.41
	Widow rockfish	36	11.01	0.99
	Pacific hake	18	3.76	0.20
	Bocaccio	16	4.60	0.38
	Greenspotted rockfish	6	1.94	0.17
	Squarespot rockfish	6	2.67	0.20
	Sebastomus group [1]	4	1.56	0.12
	Blue rockfish	3	1.46	0.16
	Unidentified fish	3	1.45	•
	Copper rockfish	1	0.46	0.08
	Flag rockfish	1	0.46	0.04
	Halfbanded rockfish	1	0.45	0.04
	Sharpchin rockfish	1	0.46	0.04
	Total	**236**	**69.20**	**4.90**
	Minimum number of species	11		
Holly	Rockfish YOY	62	20.22	0.54
	Painted greenling	33	13.75	0.53

continued

Table 6 (continued)

Platform	Species	Number	Estimated density (fish/100m^2)	Estimated biomass (kg/m^2)
Holly continued	Sharpnose seaperch	14	6.22	1.84
	Squarespot rockfish	11	4.02	0.12
	Copper rockfish	8	3.77	0.28
	Blacksmith	4	1.82	0.49
	Gopher rockfish	4	2.13	0.23
	Pile perch	4	2.23	0.99
	Widow rockfish	3	1.80	0.31
	Bocaccio	1	0.57	0.04
	Kelp rockfish	1	0.67	0.34
	Total	**145**	**57.21**	**5.70**
	Minimum number of species	10		
Grace	Widow rockfish	103	28.92	3.90
	Rockfish YOY	76	25.18	0.32
	Chilipepper	25	6.73	0.64
	Sebastomus group[1]	9	4.67	0.25
	Painted greenling	8	3.37	0.16
	Squarespot rockfish	5	1.58	0.18
	Flag rockfish	2	0.88	0.03
	Greenspotted rockfish	2	0.80	0.06
	Swordspine rockfish	2	1.09	0.08
	Calico rockfish	1	0.62	0.04
	Kelp greenling	1	0.54	0.05
	Rosy rockfish	1	0.54	0.07
	Treefish	1	0.54	0.04
	Total	**236**	**75.47**	**5.76**
	Minimum number of species	11		
Gail	Rockfish YOY	48	21.49	0.72
	Flag rockfish	10	4.92	0.40
	Widow rockfish	8	3.77	0.27
	Bocaccio	7	3.07	0.25
	Greenspotted rockfish	3	1.42	0.18
	Painted greenling	2	1.29	0.14
	Unidentified fish	2	1.10	•
	Blue rockfish	1	0.63	0.06
	Total	**81**	**37.69**	**2.00**
	Minimum number of species	6		

[1] *Sebastomus* group may include greenblotched, greenspotted, pinkrose, rosethorn, rosy, starry, or swordspine rockfishes.

alternation of warm- and cold-water conditions that have occurred over millennia (MacCall, 1996).

Previous surveys of rockfishes at the two most inshore platforms of the Santa Barbara Channel, Hilda and Hazel, provide some evidence for the plasticity of rockfish populations in the Santa Barbara Channel. In the late 1950s, Carlisle et al. (1964) found large numbers of bocaccio and olive, copper, and brown rockfishes. Most of these fishes were either YOYs or older juveniles. By 1975, olive and brown rockfishes were still abundant, but bocaccio and copper rockfishes were uncommon (Bascom et al., 1976). In this latter survey, blue rockfish, not reported by Carlisle et al. (1964), were abundant.

Thus, we believe that the relative dearth of juvenile rockfishes around Southern California Bight platforms is not a permanent condition but represents a fluctuating system. It is likely that as oceanographic conditions in the Southern California Bight become more favorable to rockfish recruitment, offshore platforms in the Santa Barbara Channel may well harbor far greater numbers of juvenile rockfishes than at present. In fact, indirect evidence implies that juvenile rockfishes were at one time

far more abundant around southern California platforms. This conclusion comes from observations we made in the mid-1970s, a period of relatively strong juvenile rockfish recruitment off California (Love and Westphal, 1990). During that period, we observed a significant recreational fishery directed at juvenile widow rockfish and bocaccio (and to a certain extent olive and blue rockfishes) at platform Holly, as well as at a number of other Santa Barbara Channel platforms. We estimate that tens of thousands of these YOY and 1- and 2-yr-old fishes were caught over the course of about three years.

The absence or relative rarity of such common nearshore species as kelp bass (*Paralabrax clathratus*), opaleye (*Girella nigricans*), black seaperch (*Embiotoca jacksoni*), and white seaperch (*Phanerodon furcatus*) from the upper waters was particularly striking. This is in contrast to the inshore platforms and reefs of this area that harbor many of these species (Carlisle et al., 1964; Ebeling et al., 1980; Schroeder[3]). A most important cause for the absence of nearshore species is the isolation of these offshore structures; relatively deep water separates them from the mainland. This distance may effectively cut these species off from source populations of many shallow-water species. Thus, it may be difficult for the young of many species to either reach these platforms or become established there. Seaperches are viviparous and produce fully developed

[3] Schroeder, D. 1997. Marine Science Institute, University of California, Santa Barbara, CA 93106. Personal commun.

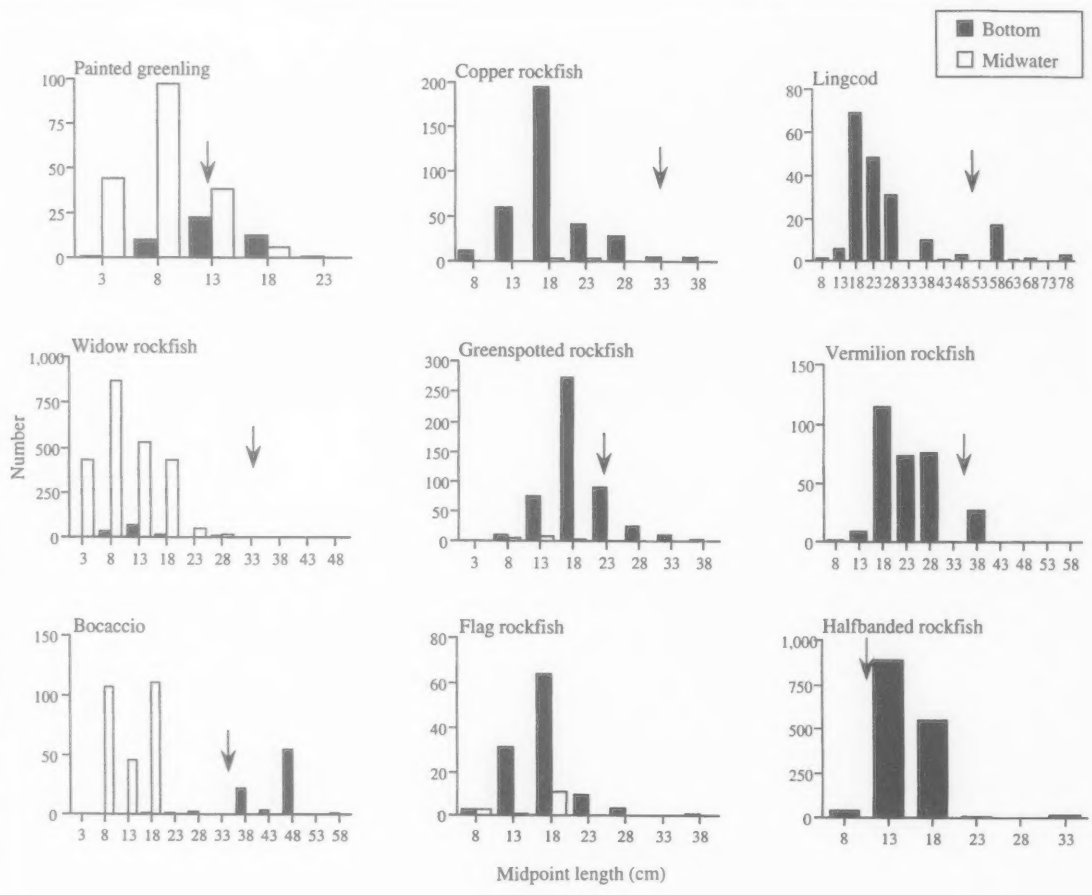

Figure 8

Length-frequency distributions of nine common species on midwater and bottom transects on all platforms combined. Midpoint length is the midpoint of 5-cm length bins.

young that do not disperse widely, making it unlikely that they commonly find their way to platforms. Kelp bass and opaleye produce pelagic larvae and although it is likely that some may settle to the platforms, conditions at these structures may preclude their survival after settlement. Young opaleye seem to require quiet intertidal waters and kelp bass YOY may need algae or thick benthic turf to avoid predation (Carr, 1994; Stephens[4]). Both of these conditions are lacking at platforms. Moreover, in the study area kelp bass recruitment is only sporadic and may not have occurred in the recent past. Thus, strong currents and lack of suitable habitat around platforms may reduce the amount of successful recruitment of these and other nearshore species.

A few species, notably painted greenling, do seem to be well adapted to a substrate-associated life in the midwaters. Judging from the very small individuals we observed, it is likely that larvae of this species recruit directly to the platform and settle out in the shallower portions. We saw a wide range of sizes, from newly settled individuals to adults, sitting on the crossbeams and hanging vertically on the pilings. Other than painted greenling, only a few juvenile flag, greenspotted, copper, swordspine, gopher, and rosy rockfishes were seen sitting on the platform in midwater.

Although juvenile rockfishes dominated the platform midwater, for some species platform bottoms tended to harbor a wider range of life stages. For some rockfishes (such as copper and greenspotted rockfishes), the entire range of stages from YOY to adults were present. In these species, the smaller individuals tended to live somewhat away from the legs and crossbeams and more among those parts of the mussel shell mounds a few meters from the platform. Although juvenile vermilion rockfish were common on several of the shallower platforms, we saw no YOYs around any of these structures. Vermilion rockfish tend to settle out in the nearshore, relatively shallow waters, and it is likely that even the shallowest of the surveyed platforms were situated in waters too deep for successful recruitment. This supposition was born out in our SCUBA diver surveys of platform Gina, located off Port Hueneme, southern California. Platform Gina is located in waters about 33 m deep. Divers have surveyed the entire structure and on several occasions have noted YOY vermilion rockfish at the bottom of the platform.

The situation with lingcod is particularly interesting. Including observations from all platforms, we observed all life stages from YOYs to large adults. However, almost all the young fish lived around platform Irene, in relatively high densities. From the lengths of these animals (Miller and Geibel, 1973), we determined that these fish were either YOYs or one-year-olds. We noted that most were sitting in the mussel shells on the bottom slightly away from the structure. In an underwater survey that encompasses seven platforms and 61 natural reefs in central and southern California, we have never encountered juvenile lingcod densities approaching the levels noted around platform Irene. Similar submersible research farther north, off Big Sur-Monterey (Yoklavich[5]) and Alaska (O'Connell[6]) also implies that such aggregations are very rare. The aggregation around Irene may also be relatively stable because we saw similar high densities in the subsequent 1997 survey. It is unclear what attracts young lingcod to this location. A large juvenile aggregation was noted off Big Sur on a sandy bottom covered with ripple marks (Yoklavich[4]). Perhaps young lingcod seek out substrate with at least some vertical relief and, at Irene, mussel shell mounds provide this type of relief.

Many bottom fishes tended to be patchily distributed around individual platforms. This is particularly true of the aggregating species, such as bocaccio and vermilion and halfbanded rockfishes. Whether this is in response to current pattern, variations in platform structure, or to other parameters is not clear at this point. We have also observed a tendency for small individuals, such as halfbanded rockfish or juvenile greenspotted rockfish, to be found away from larger, presumably predacious, individuals. Smaller fishes also tend to be found farther away from the platform, again probably to avoid the larger fishes nestled in the structure.

Fishing pressure is intense over most of the natural reefs in southern California and platforms may act as refuges for rockfishes and lingcod. An example is the relatively high numbers of bocaccio living around platform Gail. Historically, bocaccio were very important recreational and commercial fish along all of California and owing to a combination of over-fishing and poor juvenile recruitment, their populations have drastically decreased (Ralston et al., 1996). Our survey of the fish assemblages of 61 natural reefs off southern and central California shows that platform Gail has by far the highest density of adult bocaccio of all of these sites (10.5 fish/100 m^2 on platform Gail

[4] Stephens, J. 1997. Department of Biology, Occidental College, 1600 Campus Rd., Los Angeles, CA 90041. Personal commun.

[5] Yoklavich, M. 1997. Pacific Fisheries Environmental Laboratory, National Marine Fisheries Service, 1352 Lighthouse Ave., Pacific Grove, CA, 93950. Personal commun.

[6] O'Connell, T. 1998. Alaska Department of Fish and Game, 304 Lake St., Rm. 103, Sitka, AK, 99835. Personal commun.

compared with 4.4 fish/100 m^2 on the highest density natural reef). The reef was located on the northern side of the passage between San Miguel and Santa Rosa islands. The average density of bocaccio across all natural reefs surveyed in 1996 was only 1.26 fish/100 m^2. The large numbers of bocaccio around Gail may reflect the minimal fishing pressure around this platform. Fishing by recreational or commercial vessels near platforms is generally discouraged by platform operators. In addition, because larger fishes tend to live close to or inside the platforms, they are difficult to catch because the habitat close to or inside the platforms eludes most fishing gear.

We realize that the data presented in this paper represent a "snapshot" in time and thus issues of seasonality or interannual variation in assemblage structure remain to be addressed. Longer-term surveys of the fish fauna on two platforms in the Gulf of Mexico as well as one in the Santa Barbara Channel showed considerable diel and seasonal variation in the number of species present (Carlisle et. al 1964; Hastings et. al. 1975). In addition, monthly SCUBA observations on one shallow-water platform indicate that there may be large temporal changes in assemblage structure (Schroeder[2]). Despite this, the differences we observed in fish assemblages among and within platforms suggest that each platform may have unique characteristics.

There has been considerable discussion regarding the role of artificial structures in aggregation or enhanced production of marine species (or both) (Carr and Hixon, 1997). Based on this study, it appears that oil platforms may serve to do both. First, large adult fishes of several species were present on several platforms where no juveniles of those species had previously been observed, e.g. vermilion rockfish. It appears that those adults may have settled away from the platforms and migrated to them at some life stage. On the other hand, several platforms had very large numbers of very young fish that presumably settled to the platforms directly from the plankton, e.g. widow rockfish. If we assume that some of these young fishes would not have found appropriate settling habitat, then platforms, at least in the short term, do play some role in enhancing production. To ultimately assess the role of platforms in production of reef fishes, it will be necessary to understand the fate of the young fish settling to them.

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Spawning site fidelity in Atlantic bluefin tuna, *Thunnus thynnus*: the use of size-frequency analysis to test for the presence of migrant east Atlantic bluefin tuna on Gulf of Mexico spawning grounds

David Nemerson
National Audubon Society
550 South Bay Avenue
Islip, New York 11751
Present address: Marine Field Station
Institute of Marine and Coastal Sciences
Rutgers University
132 Great Bay Boulevard
Tuckerton, New Jersey 08087-2004
E-mail address: nemerson@imcs.rutgers.edu

Steven Berkeley
Oregon State University
Hatfield Marine Science Center
Oregon State University
Newport, Oregon

Carl Safina
National Audubon Society
550 South Bay Avenue
Islip, New York 11751

Abstract.—The Gulf of Mexico is the only known spawning area for bluefin tuna (*Thunnus thynnus thynnus*) in the western Atlantic. Although it is known from tag recaptures that eastern Atlantic bluefin tuna travel to the western Atlantic, whether or not these fish spawn in the western Atlantic is of critical importance in interpreting the significance of this movement. East Atlantic bluefin tuna mature at a younger age (4–5 yr) and smaller size (45 kg) than western bluefin tuna (8 yr and 135 kg), and tag recaptures indicate that some young fish make the trans-Atlantic swim. Thus the presence of small (<135 kg) bluefin tuna in the Gulf of Mexico during spawning season would constitute evidence that bluefin tuna of east Atlantic origin spawn in the west. We used size-frequency analysis to test the hypothesis that Atlantic bluefin tuna of eastern and western origins mingle on the Gulf of Mexico spawning grounds. We created a simple model to estimate the proportion of small eastern spawning fish that should be found in the Gulf of Mexico catch, assuming a 2% east-to-west transfer rate and complete mixing of eastern and western fish. Using conservative assumptions, the model predicts that between 5% and 10% of the bluefin tuna catch in the Gulf should consist of fish that are less than 135 kilograms in weight, and thus are presumably eastern migrants. We analyzed Gulf of Mexico catch records from 1980 to 1992 for the presence of bluefin tuna less than 135 kg. These small fish represented from 0% to 0.9% of the catch annually, and only 0.3% for the entire period. We conclude that eastern migrant tuna do not mix completely, if at all, with western bluefin tuna on the Gulf of Mexico spawning grounds.

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Atlantic bluefin tuna (*Thunnus thynnus thynnus*) is a highly migratory pelagic species that ranges throughout the Atlantic between 60°N latitude and the equator, although it has not been encountered south of 20°N since the 1960s. Two bluefin tuna breeding sites are known in the North Atlantic: the Gulf of Mexico and the Mediterranean Sea. No other regular spawning site has been identified in the North Atlantic (Richards, 1976; McGowan and Richards, 1989; NRC, 1994). Intensive fisheries exist for bluefin tuna along the North American and European coasts, and to a lesser degree in the high seas of the North Atlantic. Although fish tagged on both sides of the ocean have been recovered on the side opposite from their release, it is not known if bluefin tuna return to their natal spawning ground to reproduce (Turner and Powers, 1995; Cooke and Lankester, 1996). This question is of utmost importance in evaluating the significance of trans-Atlantic movement and the scale at which management must operate to be effective.

The behavior of trans-Atlantic migrating bluefin tuna is unknown, but the possibilities are bounded by two extremes. At one extreme, an emigrant may join the population on the side of the ocean to which it migrates, becoming indistinguishable from the population it joins with respect to the probability, timing, and locale of future life history events, such as maturation, spawning, and migration. At the other extreme, a migrant may always return to its natal side prior to the next spawning season.

These two extremes, and the terms used to describe them, have been the subject of some confusion in the bluefin tuna literature. The permanent transfer of individuals from one side of the Atlantic to the other has been called the "no-memory condition" (Punt and Butterworth, 1995; Powers and Cramer, 1996) or the "diffusion model" (Cooke and Lankester, 1996). The case where migrants always return to their natal side prior to the next spawning season is termed the "overlap model" by Cooke and Lankester (1996). We follow the convention of Cooke and Lankester (1996) and use the terms diffusion and overlap to refer to the two models. We use "transfer rate" to refer to the permanent transfer of an emigrant from one population to the other and "migration rate" to refer generally to the trans-Atlantic movement of individuals. Finally, we use the term "memory" to refer only to an individual's behavior with respect to spawning location, not to other life history attributes. That is, under the diffusion (no-memory) model, a migrant will spawn on the side of the ocean to which it migrates, regardless of its birth location, but will retain other life history attributes such as size or age at maturity.

The permanent transfer of individuals can be considered a migration for dispersal (Greenwood and Harvey, 1982), whereas the overlap model can be assumed to be a feeding migration, and is free of implications for reproductive mixing. One can envision intermediate scenarios combining varying degrees of memory, or philopatry. For example, migrants may remain on the opposite side for a period of years, while either participating in or foregoing spawning, before ultimately returning to their natal side. Furthermore, some migrants may exhibit spawning site fidelity while others may stray, joining previously established spawning populations (e.g. Curry, 1994).

Simulation models have shown that the dynamics of the two populations are potentially very sensitive to even low trans-Atlantic migration rates, particularly for east-to-west transfer (NRC, 1994; Porch et al., 1995; Punt and Butterworth, 1995; Powers and Cramer, 1996) because the average size of the eastern population has been about 6 to 13 times that of the western population over the past 20 years (ICCAT[1,2]; Fig. 1). Recent spawning biomass estimates for the western population are based on catches throughout the fishing area, which includes the entire North Atlantic west of 45°W longitude. If fish of eastern origin are included in these catch statistics but do not spawn in the west Atlantic, then western spawning biomass will be substantially overestimated (Powers and Cramer, 1996; American Fisheries Society[3]).

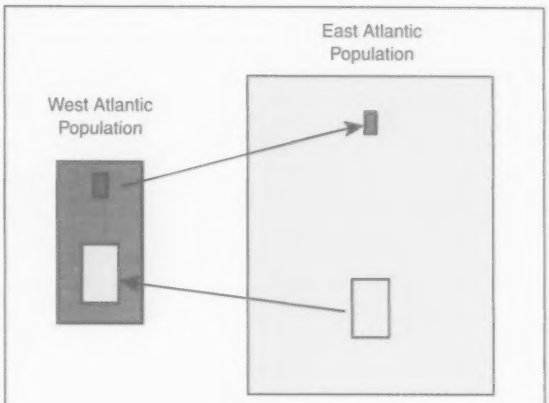

Figure 1

Representation of the effect of migration on the eastern and western populations of bluefin tuna. Migration from the larger eastern population to the west has a larger effect on the western population than does migration from the smaller western population to the east. In this schematic, the eastern population is about six times the size of the western population, and the migration rates are about 1% in each direction.

Determining the spawning site fidelity of iteroparous pelagic species that occur over a wide area of open ocean is difficult. Population differentiation can be inferred from tag-return data, comparisons of life history parameters and morphometric characters, or from genotypic variation. Several studies have attempted to analyze the population structure of Atlantic bluefin tuna with these methods (Calaprice, 1986; NRC, 1994; Cooke and Lankester, 1996).

Several investigators have reviewed and analyzed trans-Atlantic tag returns to estimate rates of migration (NRC, 1994; Punt and Butterworth, 1995; Turner and Powers, 1995; Cooke and Lankester, 1996). These studies have estimated annual migration rates of between 1% and 10% and have considered both diffusion and overlap models. Generally, these studies have sought to find interpretations of tag-return data that agree best with other estimates of population size.

[1] ICCAT (International Commission for the Conservation of Atlantic Tunas). 1994a. West Atlantic bluefin tuna. Biennial report of the ICCAT Standing Committee on Research and Statistics, 41 p. ICCAT, Estebanez Calderon 3, E-28020, Madrid, Spain.

[2] ICCAT (International Commission for the Conservation of Atlantic Tunas). 1994b. East Atlantic bluefin tuna. Biennial report of the ICCAT Standing Committee on Research and Statistics, 31 p. ICCAT, Estebanez Calderon 3, E-28020, Madrid, Spain.

[3] American Fisheries Society. 1995. Marine Fisheries Section statement on bluefin tuna, 2 p. Am. Fish. Soc., 5410 Grosvenor Lane, Ste. 110, Bethesda, MD 20814-2199.

Punt and Butterworth (1995) estimated west-to-east transfer at about 7% and east-to-west transfer at about 1.5–3%, assuming a diffusion model. They also state that the higher end of the range (3%) suggests a far larger size for the western population than do models that assume no migration. Cooke and Lankester (1996) test both diffusion and overlap models and concluded that the overlap model fits the data better. Under that model, they estimated exchange rates at 7.3% east-to-west and 9.8% west-to-east, but with no statistical difference between the two. Powers and Cramer (1996) examined the implications of a range of migration rates and degrees of spawning site fidelity. Although they made no conclusions about which scenario is most likely, they pointed out the extreme sensitivity of the results to the assumptions.

Eastern and western Atlantic bluefin tuna populations have markedly different life history parameters (Turner, 1994). The western population spawns from mid-April to mid-June (Richards, 1976). Western bluefin tuna sometimes mature as early as age 6 and are considered fully mature by age 8, at a weight of 135 kg (Baglin, 1982; NRC, 1992). The eastern population spawns from June through August (Dicenta and Piccinette, 1980) and matures at an earlier age and smaller size than the western population. Eastern bluefin tuna mature as early as age 3, at a weight of 15 kg (Rodriguez-Roda, 1967; Baglin, 1982), and are fully mature by age 5 (Rodriguez-Roda, 1967; Baglin, 1982; ICCAT[2]).

The contrast in size and age at maturity of western and eastern Atlantic bluefin tuna allows an inferential test of spawning site fidelity. Because the Gulf of Mexico is the only known spawning ground for western Atlantic bluefin tuna, and the vast majority of fish collected in the Gulf are large adults that are present only during and just prior to the spawning season (January–June), we assumed that all bluefin tuna in the Gulf during this time period are there to spawn.

If eastern Atlantic bluefin tuna that migrate to the west mature according to the eastern Atlantic maturation schedule, then the size distribution of bluefin tuna in the Gulf of Mexico should reveal the presence of eastern migrants within the western spawning population. Finding small fish (<135 kg) on the Gulf of Mexico spawning grounds would support the diffusion hypothesis and suggest that trans-Atlantic migrants from the east mix with western fish during spawning. In contrast, the absence of small fish on the Gulf of Mexico spawning grounds would imply that eastern migrants do not spawn in the west, supporting the overlap model and indicating strong spawning site fidelity. We know that small bluefin tuna from the east Atlantic swim west at least occasionally. All tagged eastern Atlantic bluefin tuna recaptured in the west have been small fish (n=19, all captured outside the Gulf), although very few large fish, and relatively few bluefin tuna overall, have been tagged in the east, compared with tagging in the west (NRC, 1994).

Methods

We analyzed the size distribution of bluefin tuna caught in the Gulf of Mexico prior to and during the spawning season (the only time of year when bluefin tuna are present in the Gulf) for fish between the known size of first breeding in the Mediterranean and the known size of first breeding for west Atlantic bluefin tuna. Any individuals smaller than the known size of first spawning in the west would presumably be of eastern Atlantic origin.

A weight-frequency distribution (WFD) of bluefin tuna on the Gulf spawning grounds was constructed by using data reported to National Marine Fisheries Service (NMFS) by the commercial fishing industry operating in the Gulf. This data set included the weight and date of capture for every bluefin tuna legally caught and landed in the Gulf. We used data from 1980 through 1992, because beginning in 1993 only bluefin tuna over 178 centimeters (70 inches) fork length were legally permitted to be retained and sold.[4]

To estimate the proportion of smaller eastern spawning fish expected at a given east-to-west annual transfer rate (i.e. fish remain with the western population), we created a simple model of the number of sexually mature eastern migrants that arrive in the west each year:

$$S^E_y = \sum P_a T^E N^E_{a,y},$$

where S^E_y = the number of age-7 or younger spawning fish of eastern origin arriving in year y;
P_a = the percentage of sexually mature adults in eastern age class a;
T^E = the east-to-west transfer rate; and
$N^E_{a,y}$ = the number in eastern age class a in year y.

East-to-west transfer was modeled as an instantaneous process that occurs prior to the spawning season. The parameter P was taken from the lit-

[4] National Marine Fisheries Service. 1995. Supplemental draft environment impact statement for a regulatory amendment for the western Atlantic bluefin tuna. U.S. Dep. Commer., NMFS, NOAA, Silver Spring, MD, 131 p.

erature, and is 0 for ages 0–3, 0.5 for age 4, and 1 for ages 5 and beyond (Baglin, 1982; ICCAT[2]). We assumed that any migrant of age 8 or greater would be the same size as western spawning fish and would not be distinguishable from western spawning fish of the same size (Cort, 1991; Turner et al., 1991; Table 1). We used a transfer rate T of 2% per year, east-to-west. This rate is at the low range of published estimates. In this initial test, we did not consider fish less than age 4 that could have migrated to the west as immature fish in prior years and then reached age 4 and maturity in the current year. Thus, our estimate of the expected number of spawning fish of eastern Atlantic origin in the western Atlantic should represent a minimum estimate and provide a conservative test for the presence of eastern migrants. $N^{E}_{a,y}$ was taken from yearly age-specific population estimates supplied by NMFS from a run of the ADAPT virtual population analysis (VPA) program with 2% east-to-west and a 1% west-to-east transfer rates, assuming no memory. Note that the population estimates from this VPA run resulted in poor fits to the indices of abundance used to tune the VPA.[5] We used these population estimates because they provided a conservative test of our assumptions.

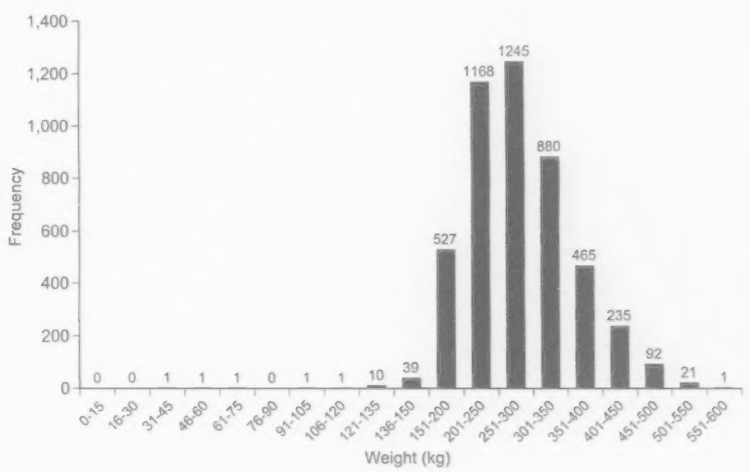

Figure 2

Weight-frequency distribution of bluefin tuna caught in the Gulf of Mexico between 1980 and 1992. The numbers over the bars indicate the total number of fish caught in the weight interval indicated.

Table 1

Estimated length at age for eastern (Cort, 1991) and western (Turner et al., 1991) Atlantic bluefin tuna.

	Length (cm)	
Age (yr)	East	West
1	53.4	48.6
2	77.0	73.8
3	98.4	97.0
4	118.0	118.5
5	135.8	138.4
6	152.1	156.8
7	166.9	173.7
8	180.4	189.4

Results and discussion

Bluefin tuna smaller than the accepted size at first spawning of western fish are very rare in the Gulf. Catches of fish less than 135 kg ranged from 0% to 0.9% of annual catch from 1980 to 1992 and averaged 0.3% over the entire period (Table 2). A complete weight frequency distribution is presented in Figure 2.

These percentages are not consistent with the low end of published migration rate estimates under the diffusion model. That is, if 2% of each age class migrated from east to west and joined the western population, we would expect to see many more small fish, i.e. fish of eastern origin, spawning in the west. (Recall that the diffusion, or no-memory model, implies that the migrant does not "remember" its natal spawning ground but does "remember" its maturation schedule.) The model predicts that between 8483 and 14,655 sexually mature migrants smaller than 135 kg would have arrived each year in 1980–92. We compared these numbers with the numbers of sexually mature fish estimated for the west from the

[5] Porch, C. 1995. National Marine Fisheries Service, Southeast Fisheries Science Center, 75 Virginia Beach Drive, Miami, FL 33149. Personal commun.

Table 2

Comparison of the number and proportion of spawning fish less than 135 kg actually caught in the Gulf of Mexico from 1980 to 1992 and predictions on the basis of a hypothesized 2% annual trans-Atlantic transfer rate. The number of small spawning fish observed is significantly less than the predicted number (χ^2=353, P<0.0001). Data in columns 2 and 3 from NMFS ADAPT VPA with 2% east-to-west and 1% west-to-east transfer rates and no memory; columns 4 and 5 are unpublished data, NMFS.

Year	Western population age 8 or greater	Predicted migrant spawning fish	Number of bluefin tuna <135 kg caught in the Gulf	Total bluefin tuna caught in the Gulf	Actual proportion of catch in the Gulf <135 kg	Predicted proportion of catch in the Gulf <135 kg
1980	97,824	10,745	0	19	0	0.099
1981	97,698	9,732	0	255	0	0.091
1982	88,781	9,402	0	228	0	0.096
1983	96,739	8,483	0	316	0	0.081
1984	92,440	8,691	0	320	0	0.086
1985	83,659	10,623	0	429	0	0.113
1986	89,444	13,151	1	395	0.003	0.128
1987	106,182	14,561	3	474	0.006	0.121
1988	117,440	14,655	3	516	0.006	0.111
1989	134,585	13,035	1	273	0.004	0.088
1990	167,115	10,631	4	469	0.009	0.060
1991	191,036	8,525	1	596	0.002	0.043
1992	260,192	9,436	1	399	0.003	0.035

ADAPT VPA run supplied by NMFS (Table 2). Table 2 shows that, if the no-memory assumption is correct and the trans-Atlantic transfer rate is at least 2%, we would expect about 5% to 10% of fish spawning in the west to be smaller than 135 kg. (Note that for this comparison to be valid, either all mature fish in the west and all small migrant spawning fish must go to the Gulf of Mexico to spawn or the same proportion of each group must go there each year.) In fact, only 0% to 0.3% of fish on the Gulf spawning grounds between 1980 and 1992 weighed less than 135 kg (Fig. 3), significantly less than predicted (χ^2=353, P<0.0001). Of 4688 fish for which NMFS has recorded weights, 15 were less than 135 kg, and 10 of those were between 120 and 135 kg (Fig. 2).

Note that this analysis considered only newly arrived migrants each year, and ignored the possible accumulation of sexually mature migrants from previous years that had not yet reached 135 kg. The continued presence of prior migrants would have raised the expected number of small spawning fish. Even without the cumulative effect of small migrants, the actual proportion of small spawning fish in the Gulf catch was about 5–10% of that predicted by the model with a 2% transfer rate. Higher transfer rates would imply that even greater numbers of small spawning fish should appear in the Gulf.

It is clear from our results that small bluefin tuna are not present among spawning fish in the Gulf of Mexico in the numbers that would be expected for even the lowest of hypothesized trans-Atlantic transfer rates. Our interpretation is that young adult bluefin tuna of eastern origin seldom or never spawn in the Gulf of Mexico and presumably do not contribute significantly to the spawning biomass of the western population. There are at least three possible alternatives: 1) eastern migrants may either delay spawning in the west until they reach 135 kg or remain in the east until they reach 135 kg, making them indistinguishable from western spawning fish; 2) migrant eastern tuna may be spawning in the west but not in the Gulf of Mexico; or 3) small migrants may be spawning in the Gulf but are avoiding detection or are being under-reported.

Size and age at maturity

If size and age at maturity are environmentally determined, then eastern migrants might follow the west Atlantic maturity schedule and thus be undetected with our methods. For example, changes in size and age at maturity may be a response to differences in interspecific or intraspecific population density. A lower population density reduces competition for food and increases per capita food intake, resulting in faster growth. When experiencing such low inter- or intraspecific population densities and enhanced growth, fish may mature at about the same size but would attain this size at a younger age (Trippel, 1995). However, for bluefin tuna, the reported dif-

ference in size and age at maturity between east and west Atlantic populations does not appear related to differences in growth rate because recent growth models indicate little difference between populations (Cort, 1991; Turner et al., 1991; Table 1).

Similarly, if differences in age or size at maturity are affected by environmental conditions, for example temperature, we would expect this effect to be manifested primarily by changes in growth rate. Again, the similarity in growth rate between east and west Atlantic bluefin tuna suggests that environmental conditions are unlikely to explain the difference in size and age at maturity.

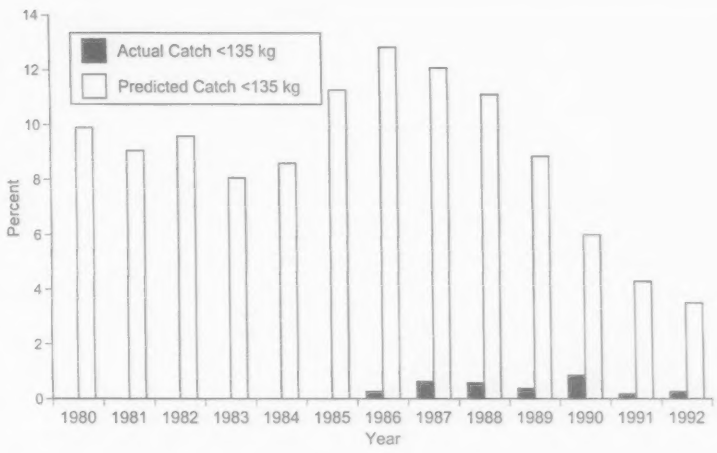

Figure 3

Comparison of actual catch of bluefin tuna less than 135 kg in the Gulf of Mexico between 1980 and 1992 and catch predicted with a 2% annual eat-to-west transfer rate and the diffusion model.

Alternatively, if age at maturity is a heritable trait, then a long period of size-selective fishing mortality could shift genotype frequencies in the population because few late-maturing fish are likely to survive to reproduce (Trippel, 1995), resulting in a younger age or smaller size at maturity, or both (Policansky, 1993; Trippel, 1995). Experiments with guppies indicate that increased mortality (as through fishing) selects for earlier maturity at smaller size (Reznick, 1993). Bluefin tuna in the east Atlantic has a longer history of exploitation and a much larger population than west Atlantic bluefin tuna. Assuming that the very large difference in population sizes results in a comparable difference in stock density, then an eastern bluefin tuna with a genetic propensity to mature at or before age 5 in the east Atlantic should, upon migrating to the west Atlantic, find itself in a relatively resource-rich, lower-density environment, which should certainly not delay maturation or inhibit spawning. Thus, although genetic effects are difficult to establish, such a large difference in size and age at maturity as between east and west Atlantic bluefin tuna is unlikely to be a result of density-dependent or environmental differences. Further, it seems unlikely that a sexually mature five- or six-year-old east Atlantic bluefin tuna would revert to immaturity upon migrating to the west Atlantic, and then remain immature for two or three years until finally spawning at age eight.

Another possible explanation for these results is that the size-at-maturity data on which this analysis depends are incorrect. In fact, Clay (1990) criticized both the Baglin (1982) and Rodriguez-Roda (1967) studies, citing small sample sizes and inadequate temporal and spatial coverage. As Clay (1990) pointed out, the Rodriguez-Roda (1967) study dealt with fish that were on their way to the spawning grounds and thus may have over-estimated the percentage of small, mature fish in the total population. Although this is a valid criticism, it is worth noting that the collection of Rodriguez-Roda also contained immature fish, implying that not all fish in his sample were on their way to the spawning grounds.

Further, although our method clearly relies on the assumption that bluefin tuna of eastern origin first spawn at a smaller size than western fish, only a small proportion of age classes 4 through 7 need be mature for our results to prevail. We investigated the sensitivity of our model to a ten-fold reduction in the maturity parameter P (i.e. to 0.05 on age-4 and 0.1 on ages 5 through 7) and found that small bluefin tuna would still be significantly rarer (chi-square test, $P<0.005$) in the Gulf than predicted under the diffusion model with a 2% east-to-west transfer rate, given our assumptions.

Finally, for the purpose of this study, we have made the most conservative assumption, i.e. that any bluefin tuna (except larvae) found in the Gulf of Mexico is spawning. Thus, it is possible that some of the smaller bluefin tuna in the Gulf of Mexico landing records were not actually spawning fish, or were of west Atlantic origin, or both.

Alternative spawning grounds

Our results might also be otabined if small migrant tuna of eastern origin spawn elsewhere in the west other than the Gulf of Mexico. A recently discovered concentration of medium and large tuna off the coast of North Carolina from January through April caused speculation that perhaps this concentration represents another spawning area. However, the lack of gonad development in a sample of seventeen fish (weighing between 65 and 183 kg) suggested that these fish were unlikely to spawn in the year they were captured and were probably immature (Belle[6]).

Several other workers have searched for evidence of bluefin tuna spawning in the west Atlantic. Mather et al. (1995) reported finding ripening small fish but no larvae. If the overlap hypothesis does prevail, then these potentially mature but nonspawning smaller fish may be eastern migrants that although capable of spawning, will return to the Mediterranean before actually doing so. McGowan and Richards (1989) reported on the sporadic presence of larvae in the Gulf Stream as far north as North Carolina but concluded that most larvae found in the Gulf Stream were either advected out of the Gulf or spawned by tuna exiting the Gulf. Furthermore, they stated that conditions are poor for larval development in the Gulf Stream and that the occasional occurrence of larvae there does not indicate an additional spawning ground. On the matter of alternative spawning grounds, the National Research Council concludes that "extensive searching has detected only two spawning localities: the Gulf of Mexico and the Mediterranean Sea" (NRC, 1994, p. 18).

Underreporting or low catchability

Two other possibilities for the lack of small bluefin tuna in the Gulf of Mexico catch are that they are present in the Gulf of Mexico and are either being caught but not recorded, or are not being caught owing to a lack of appropriate fishing effort. To test for the first possibility, we acquired records of all bluefin tuna recorded by longline observers in the Gulf of Mexico during 1993–95. Of 31 bluefin tuna recorded by observers for which actual or estimated weights were recorded, all were greater than 135 kg. We also reviewed ICCAT data for the Japanese longline fishery in the Gulf of Mexico for the period 1973 to 1981 and found that only 58 records out of 14,530 (0.4%) were for fish under 180 cm (135 kg). These data are particularly significant in light of the fact that there were no regulations concerning the retention and sale of small bluefin tuna during this period as there have been in recent years. Therefore, the Japanese would have had no incentive to intentionally misidentify or underreport small bluefin tuna. Mather et al. (1995), after reviewing longline catches in the Gulf and Caribbean prior to 1973, found only fish larger than 185 cm. They also reported very young bluefin tuna (less than 2 kg) in the Gulf of Mexico from July into November (Mather et al., 1995); fish presumably spawned a few months earlier. Similarly, Hisada and Suzuki (1982) presented length-frequency distributions of Japanese longline catches from the Gulf of Mexico which appear to show essentially no fish smaller than 200 cm.

The possibility that small bluefin tuna are present in the Gulf but are not being caught cannot be completely eliminated. However, there is a considerable accumulation of evidence that suggests that this is highly unlikely. For example, although there currently is no directed fishery for either small or large bluefin tuna in the Gulf, there is a widespread, yearround yellowfin tuna (*Thunnus albacares*) longline fishery. This fishery targets yellowfin tuna of the same size as the small bluefin tuna of east Atlantic origin that we hypothesize would be present in the Gulf if the diffusion model is correct. This fishery does have a bycatch of bluefin tuna, none of which have ever been recorded by observers as less than 175 cm.[7]

Furthermore, longline operations in the northwest Atlantic do catch small bluefin tuna, indicating that they are potentially vulnerable to this gear. Cramer and Turner[8] reported length frequencies for observer data from the U.S. longline fishery in the northwest Atlantic from 1992 to 1995, showing that over 30% of fish hooked were less than 150 cm straight fork length (Fig. 4). Similarly, catch data from the Japanese northwest Atlantic longline fishery in the 1970s and 1980s show that the catch dominated by bluefin tuna between 100 and 150 cm in several years (Fig. 8 in Hisada and Suzuki, 1982). Although failure to catch a given species or size class in an area can never rule out its presence, given the extent and diversity of fishing activity in the Gulf, it is unlikely that any significant aggregation of small bluefin tuna

[6] Belle, S. 1996. Biological sampling of bluefin tuna off Cape Hatteras, North Carolina. Final report to the New England Aquarium Corporation (NOAA requisition no. 43AANF503279), Boston, MA, 12 p.

[7] Lee, D. 1996. National Marine Fisheries Service, Southeast Fisheries Science Center, 75 Virginia Beach Drive, Miami, FL 33149. Personal commun.

[8] Cramer, J., and S. C. Turner. 1996. Standardized catch rates for bluefin tuna, *Thunnus thynnus*, from the U.S. pelagic longline fishery in the northwest Atlantic. ICCAT working document SCRS/96/69.

there would have been entirely missed.

Finally, we tested the sensitivity of our results to a range of selectivities of longline gear set in the Gulf of Mexico. With selectivities on fish smaller than 135 kg ranging from 0 to 1 (where the selectivity on fish greater than 135 kg is 1), the sensitivity analysis indicated that at selectivities greater than about 0.13, small fish were significantly less abundant (chi-square test, $P<0.05$) in the catch in the Gulf than would be expected given our assumptions and a 2% east-to-west transfer rate.

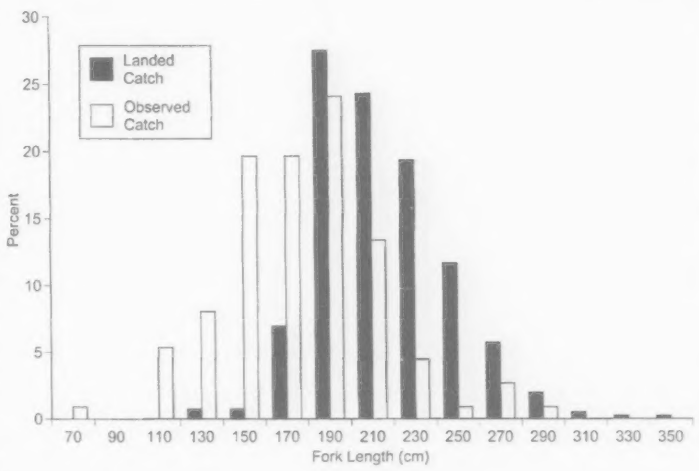

Figure 4

Length-frequency distributions of bluefin tuna caught on longlines in the northwest Atlantic (landings, n=403) and measured by observers on longline vessels (observer, n=112) between 1992 and 1995 (see Footnote 7 in the main text).

Future research on this topic must seek to address both the annual rate of trans-Atlantic movement as well as the degree of philopatry exhibited by migrants to achieve a full understanding of the population dynamics of east and west Atlantic bluefin tuna. Currently, there are studies underway to identify nuclear and mitochondrial DNA markers that may have variations specific to east and west populations (Graves et al., 1995). Examinations of otolith chemistry (microconstituent analysis) may provide information on stock differentiation and mixing rates, and researchers are currently deploying archival tags on bluefin tuna caught in the west Atlantic, primarily off Cape Hatteras, North Carolina.[9,10] These tags will record geolocation data and, if recovered, should yield a complete record of each fish's movement since its release. Finally, additional studies on the maturation schedules of fish in the east and west are still needed.

Clearly, it will take years before these studies yield sufficient information on transfer rates and philopatry to provide robust management advice. The depleted state of bluefin tuna populations worldwide, and in the west Atlantic particularly (Safina, 1993), make these issues of considerable practical importance. Until such time as these questions are answered definitively we believe that spawning site fidelity should be assumed and the stocks managed accordingly.

[9] Prince, E. 1996. National Marine Fisheries Service, Southeast Fisheries Science Center, 75 Virginia Beach Drive, Miami, FL 33149. Personal commun.

[10] Block, B. 1997. Hopkins Marine Station, Stanford University, Oceanview Boulevard, Pacific Grove, CA 93950-3094. Personal commun.

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We would like to thank Clay Porch and Dennis Lee of the Southeast Fisheries Science Center, National Marine Fisheries Service, for kindly providing assistance in acquiring the data sets that made this paper possible.

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An analysis of genetic population structure in red drum, *Sciaenops ocellatus*, based on mtDNA control region sequences

Seifu Seyoum
Michael D. Tringali
Theresa M. Bert
Department of Environmental Protection
Florida Marine Research Institute
100 Eighth Avenue SE
St. Petersburg, Florida 33701-5095
E-mail address (for S. Seyoum, contact author): Seyoum_S@epic7.dep.state.fl.us

Doug McElroy
Department of Biology
Western Kentucky University
Bowling Green, Kentucky 42101

Rod Stokes
Department of Marine Science
University of South Florida
140 7th Avenue S,
St. Petersburg, Florida 33701-5095

Abstract.—Genetic information pertaining to stock structure in red drum (*Sciaenops ocellatus*) is equivocal, complicating attempts to develop sound fishery management and stock enhancement plans. In this study, genetic stock structure was examined by using mitochondrial DNA (mtDNA) control region sequences of 209 individual red drum from six locations in the Gulf of Mexico and five locations in the nearshore Atlantic Ocean off the southeastern United States. Eighty-one polymorphic sites within a 369 base-pair portion of the control region defined 134 different haplotypes which differed by up to 26 nucleotide substitutions. Red drum showed high average within-sample haplotype (0.98) and nucleotide (0.030) diversities. Sequence divergences between pairs of haplotypes ranged from 0.27% to 7.06% ($\bar{x}$=3.17%). Cluster analysis of haplotypes revealed very little phylogeographic structure among mtDNA lineages. However, a neighbor-joining tree based on nucleotide divergence between pairs of samples showed cohesion among Atlantic samples and, to a lesser degree, among Gulf samples. In contrast to a prior study, we found no evidence that red drum in Mosquito Lagoon, Florida, constitute a self-contained, reproductively isolated population. Hierarchical analysis of molecular variance supported the hypothesis that red drum are subdivided into two weakly diverged populations with a genetic transition in south Florida between Sarasota Bay and Mosquito Lagoon. This area forms a zone of differentiation between two genetically semi-isolated populations between which the structuring of heterogeneity differs from that under the assumption of panmixia. In addition, the analysis of molecular variance also indicated that red drum from Apalachicola Bay are genetically divergent from all other samples. The Atlantic and Gulf red drum populations are likely to respond independently to harvest regulations; these fisheries should continue to be managed separately. Additional subdivision of the Gulf stock between peninsular Florida and the northern and western Gulf may also be warranted.

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Fish. Bull. 98:127–138 (2000).

Red drum (*Sciaenops ocellatus*) is a pelagic marine fish that is distributed over a large geographic range that extends throughout the northern Gulf of Mexico and along the Atlantic coast of the southeastern United States to Cape Cod, Massachusetts (Ross et al., 1983). Juveniles grow rapidly in estuarine nurseries and reach reproductive maturity by age 4. At this age they join large schools of highly dispersing adults and for the remainder of their approximately 35-year life span (Murphy and Taylor, 1990), maintain a pelagic existence except to spawn during annual congregations at the mouths of bays and estuaries. The census size of the breeding population in the Gulf of Mexico has been estimated to be greater than 7 million individuals (Nichols[1]). Abundance in the Atlantic is thought to be of a similar magnitude (Gold et al., 1993).

Red drum supports highly valuable commercial and recreational fisheries throughout its range (Mercer, 1984). Fishing pressure is directed principally on subadult year classes (ages 2–4). A high rate of annual mortality among some cohorts (Murphy and Taylor, 1990) and an overall decline in abundance and recruitment during the 1980s (Goodyear[2]) have led to concerns regarding the status of red drum spawning stocks. Because there have been no prolonged offshore fisheries for adult red drum, biological and fishery-dependent data pertaining to their spawning stocks have been limited. Therefore, fishery managers have had to rely principally on virtual population analysis and simi-

[1] Nichols, S. 1988. An estimate of the size of the red drum spawning stock using mark/recapture. Southeast Fisheries Center, Natl. Mar. Fish. Serv., Pascagoula, MS.

[2] Goodyear, C. P. 1989. Status of the red drum stocks of the Gulf of Mexico. Report for 1989. Contract no. CRD 88/89-14. Coastal Resources Div., Miami Laboratory, Southeast Fisheries Center, Natl. Mar. Fish. Serv., Miami, FL.

lar analyses (e.g. Vaughan[3]) to manage the red drum fishery. Knowledge of stock structure in red drum, i.e. the geographic relation between spawning and recruitment, is needed to facilitate the management of this fishery.

Molecular studies have been used to define appropriate geographic scales for monitoring and managing exploited animal populations (Moritz, 1994), including marine fishes (e.g. Bentzen et al., 1996; Graves, 1996; Tringali and Bert, 1996). Although several population genetic studies have been conducted on red drum, the existing data are equivocal. On the basis of significant differences in allele frequencies at a single allozyme locus, Bohlmeyer and Gold (1991) concluded that red drum are weakly subdivided between the Atlantic Ocean (North Carolina and South Carolina) and the Gulf of Mexico. However, data from other allozyme surveys (Ramsey and Wakeman, 1987; Campton[4]) did not permit the rejection of the null hypothesis that red drum form a single panmictic gene pool. In contrast, on the basis of small but statistically significant differences in the frequencies of several composite mitochondrial DNA (mtDNA) haplotypes between red drum collected from North Carolina and South Carolina waters and red drum collected from the Gulf of Mexico, Gold and Richardson (1991) and Gold et al. (1993, 1994) reasserted that red drum are weakly subdivided between these regions, ostensibly along the south Florida coast. In addition, Gold et al. (1993) reported a pattern of mtDNA differentiation in Gulf red drum consistent with the isolation by distance model (Wright, 1943) for samples ranging from Florida to Texas. However, samples from a significant portion of the species range, including locations near the putative Atlantic-Gulf division (i.e. the eastern Florida seaboard), were not assayed in any of the above studies. An equally tenable but untested hypothesis is that isolation by distance occurs over the entire range of the species, perhaps in the absence of a genetic break at a particular geographic location.

Localized population subdivision in red drum has also been postulated. From comparisons with samples from North Carolina and South Carolina waters and samples from the Gulf of Mexico, Gold and Richardson (1994) proposed that red drum inhabiting Mosquito Lagoon, Florida, form a genetically distinct population. Red drum in Mosquito Lagoon reportedly have a life history uncharacteristic of other red drum. Adults occupy this coastal lagoon throughout the year and may complete their life cycle within the lagoon (Johnson and Funicelli, 1991). Adult red drum also occur throughout the year in other coastal lagoons adjacent to Mosquito Lagoon (e.g. Banana, Indian, and Halifax Rivers), but these have not been surveyed genetically. Rather than forming a self-contained population, red drum from Mosquito Lagoon may belong to a larger subpopulation occupying Florida Atlantic waters.

Owing to the perceived decline of red drum abundance in the 1980s, state agencies in Alabama, Florida, South Carolina, and Texas studied the feasibility of stock enhancement as a means of supplementing wild populations. Hatcheries in Florida, South Carolina, and Texas currently employ stocking on a large scale (McEachron et al., 1995; FDEP[5]). Hatchery programs potentially affect the gene pools of indigenous red drum populations by way of an inappropriate introduction of non-native individuals (Hindar et al., 1991) and by hatchery-induced inbreeding effects (Tringali and Bert, 1998). For example, because broodstocks for large-scale stock enhancement programs along the Atlantic seaboard have been obtained from Mosquito Lagoon and nearby estuaries (Halstead[6]), there is a potential for artificial genetic exchange between putatively separate gene pools (e.g. those of Mosquito Lagoon and the Carolinas).

State and regional fishery managers (Vaughan[3]; FDEP[5]) and hatchery managers (FDEP[7]) have adopted the stock structure scenario proposed by Gold et al. (1993) and Gold and Richardson (1994) in which red drum are divided into Gulf of Mexico and Atlantic populations, and those fish in Mosquito Lagoon comprise a unique, self-contained Atlantic subpopulation. However, several important questions regarding the genetic structure of red drum remain unanswered; each has serious implications for fishery management and stock enhancement programs. First, are red drum populations in the Gulf of Mexico and Atlantic really subdivided or do the observed genetic differences solely reflect isolation by distance over the range of the species? Second, if a genetic break does exist somewhere between the Gulf and the coast of the Carolinas, where is it?

[3] Vaughan, D. S. 1995. Status of the red drum stock on the Atlantic coast: stock assessment report for 1995. Southeast Fisheries Science Center, Natl. Mar. Fish. Serv., Beaufort, NC, 50 p.

[4] Campton, D. E. 1992. Gene flow estimation and population structure of red drum (*Sciaenops ocellatus*) in Florida. Final report, cooperative agreement no. 14-16-009-1522, National Fisheries Research Center, U.S. Fish and Wildlife Serv., Gainesville, FL.

[5] FDEP (Florida Department of Environmental Protection). 1993. A stock assessment of red drum (*Sciaenops ocellatus*) in Florida. Florida Marine Research Inst., Dep. Natural Resources, 100 Eighth Ave. SE, St. Petersburg, FL, 24 p.

[6] Halstead, B. 1997. Stock Enhancement Research Facility, Florida Department of Environmental Protection, 14495 Harlee Road, Port Manatee, FL 34221. Personal commun.

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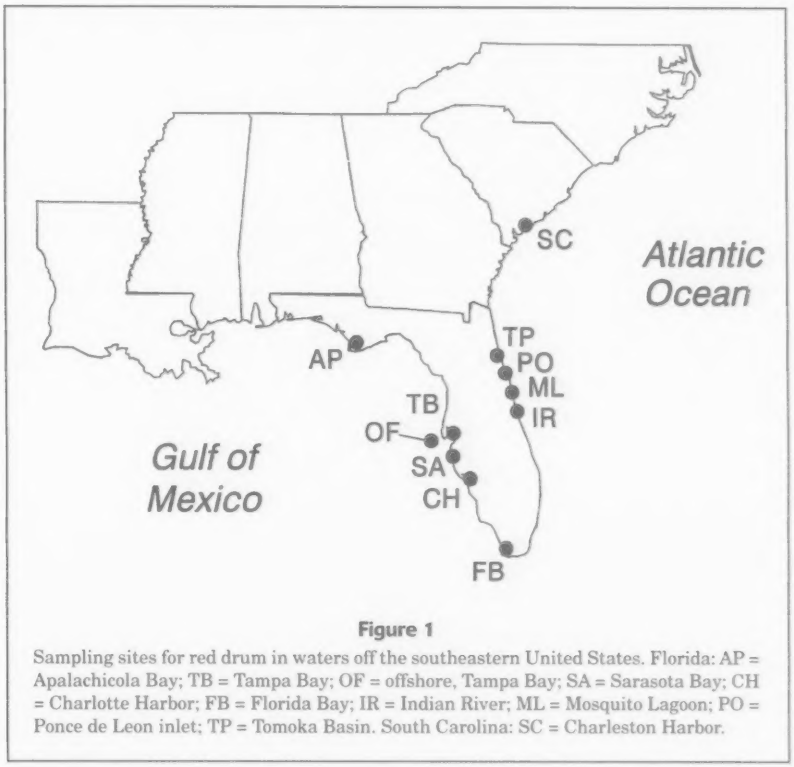

Figure 1

Sampling sites for red drum in waters off the southeastern United States. Florida: AP = Apalachicola Bay; TB = Tampa Bay; OF = offshore, Tampa Bay; SA = Sarasota Bay; CH = Charlotte Harbor; FB = Florida Bay; IR = Indian River; ML = Mosquito Lagoon; PO = Ponce de Leon inlet; TP = Tomoka Basin. South Carolina: SC = Charleston Harbor.

Third, are red drum in Mosquito Lagoon reproductively and genetically distinct or do they belong to a larger and heretofore unsampled east Florida population? To examine these questions, we obtained sequence data from the rapidly mutating mtDNA control region. Sequencing the control region has proven useful in intraspecific phylogeographic and population genetic studies of fishes (e.g. Fajen and Breden, 1992; Brown et al., 1993; Stepien, 1995; Stabile et al., 1996). We employed sampling and analytical regimes designed to test the various competing hypotheses of red drum population structure. In addition, by gathering baseline data for mtDNA control region diversity in red drum populations, we explored the potential for using the control region as a marker to assess and monitor ongoing stocking programs for wild red drum populations.

Materials and methods

Sample collection and DNA purification

Samples of red drum were collected with hook-and-line gear, trammel nets, and purse seines from riverine, estuarine, and offshore waters of the South Carolina coast (one location) and the east coast of Florida (four locations, sampled prior to stock enhancement activities), referred to collectively as the Atlantic samples; and the west coast of Florida (six locations), referred to collectively as the Gulf samples (Fig. 1). All specimens were collected between February 1992 and February 1997. Somatic muscle and liver tissue were dissected from each individual. Total length (range 280–1070 mm) of each individual was recorded prior to dissection. Tissues were frozen in liquid nitrogen and stored at –80°C in the laboratory until processing.

Approximately 100–400 milligrams of muscle or liver tissue were digested in 900 microliters of lysis buffer (0.1M Tris, pH 8.0, 0.05M ethylenediaminetetraacetid acid (EDTA), 0.2M NaCl, 1% weight by volume of sodium dodecyl sulfate (SDS), containing 1–2 milligrams of proteinase K) with moderate shaking for 3–5 h at room temperature. Following the addition of 150 microliters of chilled 8M potassium acetate, the SDS and cellular debris were precipitated for 30 min at 4°C and removed by centrifugation. Total genomic DNA was purified by phenol/chloroform extraction (Sambrook et al., 1989).

The DNA was concentrated by isopropanol precipitation, resuspended in 75 microliters of sterile water, and stored at –20°C.

mtDNA control region sequencing

Initially, we used the polymerase chain reaction (PCR; Saiki et al., 1988) and published primer sequences L15926 (Kocher et al., 1989) and H16498 (Meyer et al., 1990) to amplify a portion of the mtDNA control region of red drum. Double-stranded PCR was performed with Perkin Elmer AmpliTaq in a 50-µL reaction volume for 32 cycles in a DeltaCycler II System (Ericomp Inc., San Diego, CA), according to the methods described by Kocher et al. (1989). We amplified a 455-base-pair (bp) fragment of the control region for several individuals of red drum. However, because direct sequencing of the amplicon with the same PCR primers in the sequencing reaction yielded unsatisfactory results, we used a process of cloning and sequencing to design specific primers for red drum.

The 455-bp amplicon was cloned in pBluescript by TA cloning (Marchuk et al., 1990). Following denaturation of the plasmid DNA (Hattori and Sakaki, 1986), sequencing was done from both directions by the dideoxy termination method (Sanger et al., 1977) by using Sequenase version 2.0 (U.S. Biochemicals, Cleveland, OH) and [α-S^{35}] dATP (Dupont Biotechnology Systems, Wilmington, DE). Products of the sequencing reactions were resolved in 6% polyacrylamide/7-M urea gels that were vacuum dried at 80°C and autoradiographed with Kodak X-Omat AR film. We used the ESEE program (Cabot and Beckenbach, 1989) to align sequences. From these sequences, (Genbank accession no. AF054671), highly specific internal primers were designed for the control region of red drum. These primers partially overlapped the initial primers and were designated L15943 (5'-GTA AACCGGATGTCGGGGGTTAG-3') and H16484 (5'-GGAACCAGATACCAGGAATAGTTCA-3').

We used these custom primers to amplify a portion of the control region for 209 individuals in 50-µL reaction volumes. The PCR products were run on 1.2% low-EEO (Fisher Scientific, Norcross, GA) agarose gel during electrophoresis. The resulting bands were excised and then purified with GeneClean (Bio 101, La Jolla, CA). Double-stranded sequencing was conducted as described by O'Foighil et al. (1996).

Data analyses

Base composition, number of transitions (TSs), and number of transversions (TVs) were determined by using MEGA 1.01 (Kumar et al., 1993). Further analysis of base substitutions was conducted as in Brown and Clegg (1983). Each different haplotype was assigned a number, and the distribution of the different haplotypes was determined for each sample.

We used MEGA to generate a pairwise matrix of sequence divergence values between pairs of haplotypes and to construct an unrooted neighbor-joining tree; 200 replicates were used to estimate bootstrap values for the nodes. Sequence divergences were computed by using the pairwise-deletion option in MEGA; this distance estimator excludes sites at which indels occur on a pairwise basis. Haplotype and nucleotide diversity within samples and nucleotide divergence (D) between pairs of samples were estimated according to Nei and Tajima (1981) and Nei (1987) by using the DA option of REAP 4.0 (McElroy et al., 1992). The nucleotide divergence values were clustered by using the NJTREE program (Jin and Ferguson, 1990) based on the neighbor-joining method of Saitou and Nei (1987).

Geographic structuring of molecular variance among samples was examined by using the matrix of sequence divergences between all pairs of haplotypes in AMOVA 1.55 (Excoffier et al., 1992). In this analysis, the haplotype correlations (ϕ statistics) and their variance components were estimated in a hierarchical fashion: between regions, among samples within a region, and among individuals within samples. Statistical significances of ϕ values were computed by performing randomization tests with 500 replicates. Gold et al. (1993) concluded that red drum was subdivided into Atlantic and Gulf of Mexico populations. To determine the validity of this conclusion, we examined the spatial partitioning of molecular variance as follows. The between-region component of variance and ϕ_{CT} was first calculated for red drum samples divided into Atlantic (SC, TP, PO, ML, and IR) and Gulf (FB, CH, SA, OF, TB, and AP) regions. The compositions of the two groups were then adjusted by sequentially adding Atlantic samples to the Gulf group and then sequentially adding Gulf samples to the Atlantic group. After each addition, the apportioning of molecular variance between the resulting groups was recalculated. For example, IR (the Atlantic sample closest to the Gulf) was added to the Gulf group and tested against the remaining Atlantic samples (ML, PO, TP, and SC). ML (the second closest sample) was then added to the Gulf-plus-IR group; that grouping was then tested against PO, TP, and SC. This process was repeated until only a single sample remained in one group.

Finally, to test for an association between interpopulation D values and geographic distance (isolation-by-distance), we performed the Mantel test (BIOMstat, version 3.0; Rohlf and Slice, 1995) for samples grouped

by region (Atlantic or Gulf) and for all samples combined. The statistical significance of the association was tested by random permutation analysis by using 500 replicates (Sokal and Rohlf, 1995).

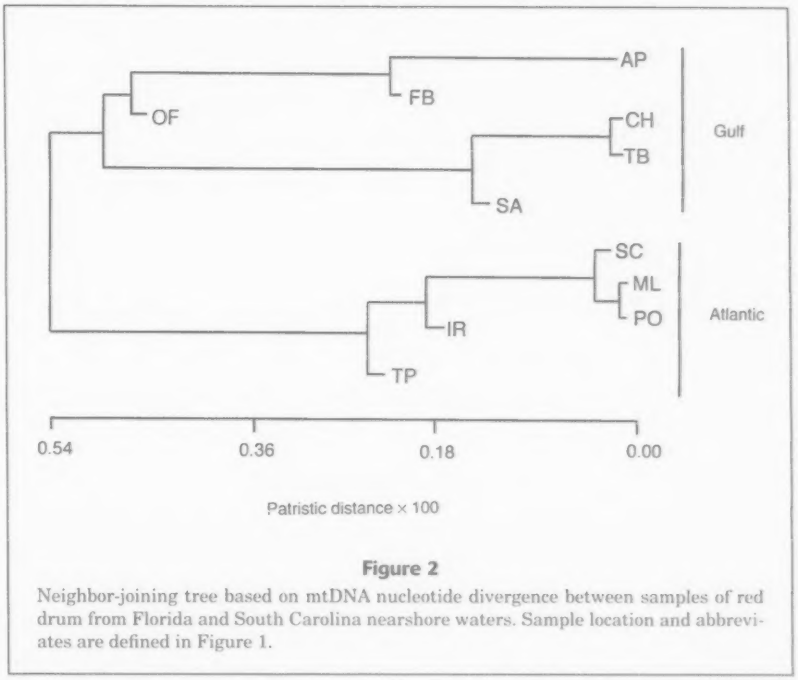

Figure 2

Neighbor-joining tree based on mtDNA nucleotide divergence between samples of red drum from Florida and South Carolina nearshore waters. Sample location and abbreviates are defined in Figure 1.

Results

We analyzed sequence data from a 369-bp portion of the mtDNA control region for 209 red drum. A total of 81 polymorphic sites were observed among all individuals. Of these, 67 sites had single-state, 11 had double-state, and 3 had triple-state transformations, totaling 98 polymorphisms and including two indels. The first indel consisted of an insertion of a pyrimidine (T or C) at position 160 and occurred in 10 individuals; the second indel consisted of a deletion of a purine (A) at position 210 and occurred in one of these individuals (Table 1). Seventy-three of the substitutions were TSs and 22 were TVs. As with control regions of other fishes (Stepien, 1995), the relative frequencies of the four nucleotide bases differed; adenine was the most prevalent (39%), followed by thymine (27%), cytosine (23%), and guanine (11%). The TS:TV ratio was 3.4:1 and was similar to ratios reported for marine and freshwater fishes (Stepien, 1995; but see Brown et al., 1993).

We observed 134 different haplotypes in the 209 individuals sequenced (Table 1). Sequences of these haplotypes have been deposited in GenBank under the accession numbers AF054672–F054805. Twenty-nine haplotypes were shared by more than one individual. Two haplotypes, no. 56 and no. 83, were shared by nine and nineteen individuals, respectively, which were widely dispersed among seven samples. Twenty-six haplotypes occurred infrequently, 25 in two to five individuals scattered among two to five samples, and one in two members of a single sample. Of the 19 individuals with haplotype no. 83, 17 were from the Atlantic and two from the Gulf. The percentage of different haplotypes in any one sample varied from 76% to 100% ($\bar{x}$=87%). Haplotype diversity within samples ranged from 0.95 to 1.00 ($\bar{x}$=0.98, SE=0.00) and nucleotide diversity ranged from 0.025 to 0.037 ($\bar{x}$=0.030, SE=0.003).

Percent sequence divergences between pairs of different haplotypes ranged from 0.3% to 7.1% ($\bar{x}$= 3.2%; SE=0.017). Between any two different haplotypes, the number of nucleotide differences varied from one to 26 ($\bar{x}$=12). The topology of the unrooted tree neighbor-joining (not shown) revealed that the 134 haplotypes were not phylogeographically structured. Haplotypes observed in Gulf and Atlantic samples were scattered throughout the tree; with the exception of two terminal groupings, nodes on the tree had low statistical support. Internal branch lengths of the tree were generally short; however, one interior branch was relatively long, and it defined the only well-supported major clade (bootstrap value=85). This clade consisted of 23 haplotypes, including the 10 haplotypes that had the insertion at position 160. The 10 insertion-bearing haplotypes were found in nine Atlantic individuals but in only one Gulf individual.

The D values between pairs of samples ranged from –0.08% to 0.10%. In the neighbor-joining cluster analysis, cohesion of the samples within geographic regions was generally observed (Fig. 2). All Atlantic samples formed a distinct clade which was separated by the longest branch of the tree from the clade formed by the Gulf samples. Less cohesion was observed among the Gulf samples, although the geographically proximal SA, TB, and CH samples clustered closely together.

Table 1

Distribution of red drum mitochondrial DNA control region haplotypes from 11 locations. Abbreviations for sample locations are defined in Figure 1.

	Gulf						Atlantic				
Haplotype	AP	TB	OF	SA	CH	FB	IR	ML	PO	TP	SC
1	1										
2	3					1					
3	1						1		1	1	
4	1										
5	1										
6	1								1		
7	1		1		1	1			1		
8	1										
9	1										
10	1										
11	1										
12	1										
13	1										
14	1										
15	1										
16	1					1					
17	1										
18	1										
19		1									
20		1									
21[1]		1									
22		1									
23		1									
24		1									
25		2									
26		1									
27		1									
28		1									
29		1									
30		1									
31		1									
32			1							1	
33			1								1
34			1								
35			1								
36			1								
37		1	1								
38			1								
39			1								
40			1								
41			1								
42				1							
43				1							
44				1							
45				2							
46				1							
47				1							
48				1							
49					1						
50					1						
51					1						
52					1						
53					1	2			1	1	
54					1						

continued

Table 1 (continued)

	Gulf						Atlantic				
Haplotype	AP	TB	OF	SA	CH	FB	IR	ML	PO	TP	SC
55		3			3	1					
56		2			1	1	1	1		2	1
57		1			1						
58					1			1			
59					1						
60					1						
61					1					1	
62					1						
63			1		1						
64					1						
65					1						
66						1					
67						1					
68						1					
69						1					
70						1					
71						1					
72			1			1			1		
73						1			1		
74						1					
75						1					
76						1					
77						1					
78						1					
79							1				
80							2		1		1
81							2				1
82							1				
83		1	1				3	5	3	3	3
84[1]							1			1	
85							1				
86							1		1	1	1
87							1				
88							1				
89[1]							1				
90								1			
91								1			
92								1			
93[1]								1			
94								1			
95[1]								1			
96								1			
97								1			
98								1			
99								2			
100								1			
101[1]								1			
102								1			
103									1		1
104									1		
105									2		2
106									1		
107									1		
108									1		
109									1		
110									1		

continued

Table 1 (continued)

	Gulf						Atlantic				
Haplotype	AP	TB	OF	SA	CH	FB	IR	ML	PO	TP	SC
111									1		
112									1		
113										1	
114										1	
115										1	
116										1	
117[1,2]										1	
118										1	
119										1	
120										1	
121										1	
122[1]										1	
123										1	
124										1	1
125											1
126											1
127											1
128											1
129											1
130[1]											1
131											1
132											1
133											1
134											1
Total	20	22	14	8	20	20	17	21	22	23	22

[1] Haplotypes with an insertion at position 160.
[2] Haplotype with an insertion at position 210.

The D value between the ML sample and the remaining pooled Atlantic samples was –0.05%. In contrast, the D value between the ML sample and the Gulf samples ranged from –0.02% to 0.076%.

The analysis of molecular variance (AMOVA) for all samples yielded a ϕ_{ST} value of –0.001, indicating that no significant heterogeneity was detected between any two samples. For the geographic analysis, in which samples were divided into shifting regional subsets, the variance components among samples within groups and among individuals within samples were not significant for any grouping. Significant values of ϕ_{CT} were observed in five of the 10 groupings (Table 2). In four of the five significant groupings, the division occurred in peninsular south Florida. Overall, results of the AMOVA suggest that a genetic transition occurs in red drum along the Florida coast between Sarasota Bay in the Gulf and Mosquito Lagoon in the Atlantic. However, the highest ϕ_{CT} value and between-group variance component were observed when samples were grouped according to their actual Atlantic and Gulf locations (Table 2, grouping 1). The significant ϕ_{CT} value for the AP sample versus all other samples suggests that an additional genetic discontinuity occurs in Gulf waters off northwest Florida.

In the Mantel test between interpopulational D and geographic distance, no association was observed among the Atlantic samples (P=0.20) or among the Gulf samples (P=0.053). However, a significant association was observed for all red drum samples (P<0.01), reflecting the genetic transition that occurs in south Florida.

Discussion

Genetic population structure

Because there are few absolute barriers to gene flow in the ocean, it is generally expected that marine species with continuous distributions, large populations, and high levels of larval and adult dispersal should have very little intraspecific population structure over large geographic areas (Avise, 1987; Palumbi, 1992).

Table 2

Geographic analysis of molecular variance in the mtDNA control region of red drum. The table lists the ϕ_{CT} values and between-group variance components for the ten possible geographical groupings and the probability P of finding a more extreme variance component by chance (500 permutations). In each group, the letter G represents samples from the Gulf of Mexico (G_1=AP, G_2=TB, G_3=OF, G_4=SA, G_5=CH, G_6=FB) and the letter A represents samples from the Atlantic Ocean (A_1=IR, A_2= ML, A_3=PO, A_4=TP, A_5=SC). Abbreviations for sample locations are defined in Figure 1.

Grouping	First group	Second group	Variance between groups (%)	Φ_{CT}	P
1	$G_1G_2G_3G_4G_5G_6$	$A_1A_2A_3A_4A_5$	1.96	0.020	<0.002
2	$G_1G_2G_3G_4G_5G_6A_1$	$A_2A_3A_4A_5$	1.40	0.014	0.012
3	$G_1G_2G_3G_4G_5G_6A_1A_2$	$A_3A_4A_5$	0.39	0.004	NS
4	$G_1G_2G_3G_4G_5G_6A_1A_2A_3$	A_4A_5	–0.17	–0.002	NS
5	$G_1G_2G_3G_4G_5G_6A_1A_2A_3A_4$	A_5	–0.54	–0.005	NS
6	$G_1G_2G_3G_4G_5$	$G_6A_1A_2A_3A_4A_5$	1.48	0.015	0.004
7	$G_1G_2G_3G_4$	$G_5G_6A_1A_2A_3A_4A_5$	0.78	0.008	0.044
8	$G_1G_2G_3$	$G_4G_5G_6A_1A_2A_3A_4A_5$	0.73	0.007	NS
9	G_1G_2	$G_3G_4G_5G_6A_1A_2A_3A_4A_4$	0.74	0.007	NS
10	G_1	$G_2G_3G_4G_5G_6A_1A_2A_3A_4A_5$	1.50	0.015	<0.002

Red drum is a pelagic marine fish with these demographic and life history characteristics. However, although some genetic exchange may occur between red drum from distant locations, populations differ from the expectation of genetic homogeneity. Hierarchical analysis of the structuring of genetic variance supports the hypothesis that the species is weakly subdivided between the Atlantic Ocean and the Gulf of Mexico. The existence of the subdivision is also supported by cluster analysis of sequence divergence values. The geographic coverage of our samples, particularly the inclusion of samples from Florida's eastern seaboard, allowed us to infer that the genetic break separating these two populations occurs in south Florida. Another genetic discontinuity apparently occurs in Gulf waters off northwest Florida.

The Atlantic-Gulf subdivision in red drum may result from a combination of extrinsic and behavioral factors. Gold et al. (1993, 1994) summarized a number of potentially important oceanographic and geographic factors. Because the southernmost portion of the east Florida shelf is extremely narrow and provides little of the neritic habitat (Jones et al., 1985) generally occupied by adult red drum, it may represent a significant barrier to adult migration. Biotic factors largely preclude large-scale passive dispersal of eggs and larvae (Peters and McMichael, 1987), and widespread dispersal at the juvenile stage is rare (Murphy and Taylor[8]). If partitioning of genetic variation in red drum results from adult migration or vagrant movement between spawning locations, it is not evident from studies of fish movement. Although very few tagged adult red drum have been recaptured after spending significant periods at large, the evidence suggests that some are highly mobile and may disperse to distances of up to 320 km (Woodward and Nicholson, 1997; Crabtree[9]). However, movement of red drum between the Atlantic and Gulf regions has not been documented. In apparent contrast to the mark-recapture data, recent ultrasonic tracking studies of adults provide limited evidence for spawning fidelity to certain Atlantic estuaries over two-to-three-year periods, and potentially longer (Nicholson and Jordan[10]). Overall, the available movement data for adult red drum are not adequate to draw conclusions relating to regional recruitment processes and patterns of dispersal. Nevertheless, it is clear from the genetic data that reproductive exchange between spawning populations in the Atlantic and Gulf regions is limited.

Because red drum from Mosquito Lagoon were used to produce hatchery populations for at least two stock enhancement programs along the Atlantic seaboard, it was important to determine whether the spawning aggregation within that system represented a self-contained, genetically divergent popu-

[8] Murphy, M. D., and R. G. Taylor. 1989. Tag/recapture and age validation of red drum in Florida. Final report, NOAA grant NA86-WC-H-06136, National Marine Fisheries Service, Pascagoula, MS, 27 p.

[9] Crabtree, R. 1997. Florida Marine Research Institute, Department of Environmental Protection, St. Petersburg, FL. Unpublished data.

[10] Nicholson, N., and S. R. Jordan. 1994. Biotelemetry study of red drum in Georgia, November 1989–June 1993. Coastal Resources Division, Georgia Department of Natural Resources, Brunswick, GA, 65 p.

lation. Contrary to the study of Gold et al. (1994), we found no evidence to support the hypothesis that red drum in Mosquito Lagoon are reproductively isolated from other Atlantic red drum. In our survey of the mtDNA control region, there was no nucleotide divergence between Mosquito Lagoon and other Atlantic samples, whereas divergence values between the Mosquito Lagoon and the Gulf samples were among the highest. Furthermore, other investigators also found no significant differences at the allozyme loci that putatively distinguish Mosquito Lagoon red drum from other red drum (Campton[4]; Crawford and Bert[11]). This lack of difference suggests that the allozyme frequency differences observed by Gold and Richardson (1994) may not be temporally stable. A lack of samples geographically proximal to Mosquito Lagoon may have also influenced the outcome of that study. Considering all the evidence, it seems more likely that red drum from Mosquito Lagoon belong to the larger genetic population occupying nearshore Atlantic waters of the southern United States. Although it is generally better to obtain hatchery broodstock from locations within or near the intended release site (Utter, 1998), the use of Mosquito Lagoon red drum as a source of broodstock in Atlantic coast stock enhancement programs should not produce a negative genetic impact on wild red drum.

Implications for fishery and hatchery management

Because red drum support valuable fisheries throughout the Gulf of Mexico and Atlantic seaboard, the species is of special concern to state and regional fishery management agencies. Our results support the hypothesis that a genetic transition in red drum population structure occurs in south Florida. In theory, it requires the regular exchange of only a few individuals between breeding populations to homogenize their genetic composition (Slatkin, 1987). Thus, genetic exchange between Atlantic and Gulf stocks by any recruitment process must be sufficiently low to allow genetic differences to accumulate or, if the differences reflect a historical disassociation, for them to be maintained. The two red drum stocks can best be described genetically as demes, separate semi-isolated groups between which the structuring of heterogeneity differs from the assumption of panmixia (Hartl and Clark, 1989). Therefore, the Atlantic and Gulf stocks are likely to respond independently to harvest regulations and these fisheries should continue to be managed separately.

[11] Crawford, C., and Bert, T. 1997. Florida Marine Research Institute, Department of Environmental Protection, St. Petersburg, FL. Unpublished data.

Gold et al. (1993) observed a pattern of isolation by distance in the distribution of mtDNA haplotypes among Gulf samples that ranged from the southeast coast of Texas to the southwest coast of Florida. Although we did not observe a similar pattern for Gulf samples ranging from Apalachicola Bay to Florida Bay, the probability value for the Mantel coefficient was nearly significant at the 0.05 level and the AMOVA value for the Apalachicola sample versus all other samples was highly significant. This indicates that the minimum geographic scale at which the isolation-by-distance mechanism operates is greater than the distance between Apalachicola Bay and Florida Bay (approximately 670 km) or that genetic discontinuity also exists between Florida Gulf red drum and red drum inhabiting the northern and western Gulf of Mexico. Accordingly, cooperative management of the Gulf fishery on a regional basis is appropriate. No pattern of isolation-by-distance was evident for red drum along the southern Atlantic seaboard; the fishery between South Carolina and southeast Florida should be managed as a single unit.

Our principal objective for undertaking this study was to improve upon available genetic information relating to red drum population structure for fishery management purposes. Our most informative statistical tools were those that assessed relationships among the samples. Genotype frequency differences accumulate quickly in subdivided populations compared with the rate at which distinct phyletic lineages emerge and sort geographically. Moreover, mitochondrial DNA restriction fragment length polymorphism and sequence data for marine populations are typically characterized by haplotype distributions which consist of a few numerically and geographically prevalent haplotypes and many rare, geographically restricted haplotypes that may be important with respect to population structure. Therefore, as our results and the results of Tringali and Bert (1996) demonstrate, genetically-based management units (*sensu* Moritz, 1994), especially in marine fishery stocks, may be more easily identified by applying population-level analyses that take full advantage of both differences in genotype frequencies among samples and phylogenetic relatedness of individual genotypes. Statistical tests of association when applied to skewed haplotype distributions often lack the power to detect the low levels of population divergence that may characterize marine populations. Moreover, these tests ignore the interrelatedness of haplotypes in terms of sequence similarity or difference. The two principal analytical methods we employed, clustering of intersample nucleotide divergence values and AMOVA, are based on both the occurrence of haplotypes at particular locations and their sequence similarity to other

haplotypes at those locations. This approach may be particularly important in studies of pelagic marine species because, like red drum, genetic divergence separating populations is often buried within a high background of overall genetic diversity.

Finally, Moritz (1994) described the potential of using mtDNA as a marker for evaluating the success of stock enhancement programs. For this application, the mtDNA haplotypes borne by hatchery fish should be sufficiently rare in wild populations. The portion of control region we examined provides an excellent source of naturally occurring genetic markers. Because the percentage of wild red drum individuals with different haplotypes in any given sample averaged 87% and ranged up to 100%, a genetic monitoring program using this mtDNA fragment to track haplotypes borne by hatchery-released red drum after release should allow for assessment of the survival and reproductive output of these fish in the natural environment. In addition, nucleotide substitutions in this portion of the control region could be used to estimate the contribution of each female parent to the broods. This information will be important in the evaluation of breeding protocols designed to optimize levels of genetic variability in hatchery broods, in the assessment of genetic risk posed to wild red drum populations by hatchery stocking programs (e.g. Tringali and Bert, 1998), and in the evaluation of stock supplementation programs.

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Mortality and hematology of blue crabs, *Callinectes sapidus*, experimentally infected with the parasitic dinoflagellate *Hematodinium perezi**

Jeffrey D. Shields
Christopher M. Squyars
Department of Environmental Sciences
Virginia Institute of Marine Science
The College of William and Mary
P.O. Box 1346, Gloucester Point, VA 23602
E-mail address (for J. D. Shields): jeff@vims.edu

Abstract.—On the eastern seaboard of the United States, populations of the blue crab, *Callinectes sapidus*, experience recurring outbreaks of a parasitic dinoflagellate, *Hematodinium perezi*. Epizootics fulminate in summer and autumn causing mortalities in high-salinity embayments and estuaries. In laboratory studies, we experimentally investigated host mortality due to the disease, assessed differential hematological changes in infected crabs, and examined proliferation of the parasite. Mature, overwintering, nonovigerous female crabs were injected with 10^3 or 10^5 cells of *H. perezi*. Mortalities began 14 d after infection, with a median time to death of 30.3 ±1.5 d (SE). Subsequent mortality rates were greater than 86% in infected crabs. A relative risk model indicated that infected crabs were seven to eight times more likely to die than controls and that decreases in total hemocyte densities covaried significantly with mortality. Hemocyte densities declined precipitously (mean=48%) within 3 d of infection and exhibited differential changes in subpopulations of granulocytes and hyalinocytes that lasted throughout the course of the infection. Crabs that did not present infections after injection (i.e. "immune" hosts) did not show hemocytopenia and exhibited significant long-term (21–27 d) granulocytemia. Detection of the parasite in the hemolymph of infected crabs increased from approximately 30% after 14 d to 60% after 21 d to 100% after 35 d. Plasmodial stages were, however, detectable in histological preparations of the heart within 3 days of infection and increased in number over 5 and 7 days. Sporulation of the parasite occurred over a short time (at least 4 d, after 43 d of infection) and did not culminate in the immediate death of the host. *Hematodinium perezi* represents a significant threat to the blue crab fisheries in high-salinity estuaries. Although the parasite infects male and female crabs, it may have a greater impact on mature females as they move to higher salinities to breed.

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Hematodinium perezi is a parasitic dinoflagellate that proliferates in the hemolymph of several crab species. In the blue crab, *Callinectes sapidus*, *H. perezi* is highly pathogenic and usually kills the host. The main symptom of the infection is lethargy. Heavy infections are characterized by discolored (brown, yellow, milky or chalky) hemolymph that does not clot. The disease occurs in blue crabs in high-salinity (>11‰) waters from Delaware to Florida, and in the Gulf of Mexico (Newman and Johnson, 1975; Messick and Sinderman, 1992). In 1975, Newman and Johnson (1975) reported a prevalence of 30% in blue crabs from Florida; the effect of this disease on the blue crab population was thought to be high.

In 1991 and 1992, prevalences of infection up to 100% were found in blue crabs (mean prevalence=43%, several locations from 70% to 100%, n=971) from coastal bays in Maryland and Virginia (Messick, 1994). Commercial watermen reported reduced catches, lethargic and moribund crabs in pots and shedding facilities, and crabs that died soon after capture (Rux, Oesterling[1]). In 1996 and 1997, 10% to 40% of adult crabs from the eastern portions of Chesapeake Bay in Virginia were infected.[2] The disease has a low prevalence or does not occur in the larger, riverine ("bayside") fishery; it appears most detrimental to the coastal ("seaside") crab fisheries.

Outbreaks of infestation by *Hematodinium* spp. have caused concerns to several major crustacean fisheries. Significant population declines and economic losses have been reported for the Tanner (*Chionoecetes bairdi*) and snow (*C. opilio*) crab fisheries of Alaska and Newfoundland (Meyers et al., 1987, 1990; Taylor and Khan, 1995),[3] the Norway lobster (*Nephrops norvegicus*) fishery of western Scotland (Field et al., 1992), and the velvet crab (*Necora puber*) fishery of western France (Wilhelm and Miahle, 1996). The parasite causes a condi-

* Contribution 2241 from the Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23602.

[1] Rux, S. 1993. Red Bank Seafood Co., Box 37 Marionville, VA 23408. Personal commun.; Oesterling, M. 1993. VASG, Virginia Inst. Marine Science, Gloucester Point, VA 23062. Personal commun.

[2] Shields, J. D. 1997. An investigation into the epizootiology of *Hematodinium perezi*, a parasitic dinoflagellate in the blue crab, *Callinectes sapidus*. Saltonstall-Kennedy Program, National Marine Fisheries Service, NOAA. Final Report.

[3] Prevalences in Newfoundland are now at 1–15% in the northern bays. Taylor, D. 1998. DFO, CP 5567, White Hills, St. Johns, Newfoundland, Canada, A1C 5X1. Personal commun.

tion known as bitter crab disease in snow and Tanner crabs (Meyers et al., 1987). Low prevalences (1–4%) of another species, *H. australis,* have been reported in sand (*Portunus pelagicus)* and mud (*Scylla serrata)* crabs from Australia (Shields, 1992; Hudson and Shields, 1994).

Infections of *Hematodinium* spp. or *Hematodinium*-like species have been reported from a variety of different hosts (see Shields, 1994, for review). There are, however, only two described species of *Hematodinium*: *H. perezi* Chatton and Poisson, 1931, and *H. australis* Hudson and Shields, 1994. By convention (Newman and Johnson, 1975; MacLean and Ruddell, 1978) and from its distinct morphological features, we concur that *Hematodinium perezi* is the infectious species in the American blue crab.

Blue crabs sustain one of the largest fisheries in Chesapeake Bay. Current management plans and state regulations are based on population assessments that include numbers of juvenile and adult crabs found during winter, spring, and summer surveys (Lipcius and Van Engel, 1990; Abbe and Stagg, 1996; Rugolo et al., 1998). Although these projections include estimates of natural mortalities, they do not account for the potential epizootics and mortalities caused by *Hematodinium perezi.* In this study, we examined host mortality in controlled laboratory experiments and documented changes in the hemograms (total cell counts, and differential counts) of inoculated crabs versus uninfected crabs. We also examined proliferative growth of *H. perezi* at approximately weekly intervals and made observations on the biology and life history of the parasite.

Materials and methods

Blue crabs were collected from Chesapeake Bay and several of its subestuaries during the annual VIMS Winter Dredge Survey (part of the Chesapeake Bay Stock Assessment Program) with a 1.83-m-wide Virginia crab dredge fitted with 0.5-inch (1.25-cm) Vexar mesh dragged on the bottom for one minute at three knots. Crabs were also taken with commercial crab pots from two reference locations on the Delmarva Peninsula, Red Bank and Hungars Creeks, Virginia. Uninfected crabs were housed together for three to seven days prior to treatment to ensure acclimation and absence of overt bacterial or protozoal diseases (as assayed below). During the experiments, crabs were fed fish and squid semiweekly and held individually in aquaria (5 gal., 19 liter) at 20° to 21°C, and 24 ppt salinity. Although *H. perezi* infects both sexes, only mature, nonovigerous female crabs (healthy, orange maturing gonads, little to no shell damage, 120–160 mm carapace width including epibranchial spines) were used in the experiments. Females were used to limit the number of treatment effects (e.g. potential differences between sexes) and to improve sample sizes given the laborious nature of the experiments.

Hematodinium perezi was maintained in the laboratory by serial passage of infected hemolymph. Hemolymph from naturally infected crabs was injected directly into uninfected crabs. Naïve (unexposed) crabs and crabs used for inoculation experiments were obtained from low-salinity non-enzootic locations. Infected and inoculated crabs were housed separately and used as hemolymph donors to inject naïve hosts (10^5–10^6 parasites per host). Injections were given in the arthrodial membrane of the fifth leg at the juncture of the basis with the carapace. We have maintained *H. perezi* for over seven months using this method with no apparent loss from pathogenicity.

Two mortality experiments and one early life history experiment were undertaken. The mortality-I experiment used raw, infected hemolymph as the inoculant. Although appropriate for maintaining infections in the laboratory, raw hemolymph cannot be adjusted to manipulate parasite densities without the use of physiological buffers, nor can it be guaranteed as sterile without appropriate assessment (see Welsh and Sizemore, 1985). Preliminary experiments with sterile sea water, physiological buffers, and infected hemolymph indicated that buffer-washed parasites remained infectious, and could, therefore, be adjusted to consistent densities appropriate to controlled experiments. The mortality-II experiment used buffer-washed parasites adjusted to a density similar to that used in the mortality-I experiment. Mortality-II experiment closely resembled mortality-I experiment except for 1) handling (buffer washes with centrifugation) and 2) the use of plasmodial versus uninucleate stages of the parasite. Uninfected crabs served as controls in both experiments. Controls were used to assess handling effects and to establish baseline densities of hemocytes. The early infection experiment was designed to examine the effects of early infections on the hematology of the host and the early life history of the parasite. Experimental densities in the early infection experiment were four times higher than those in the previous experiments (4.1×10^5 vs. approx. 1.0×10^5 parasites/crab, respectively) and were arbitrarily higher to insure observation of parasites prior to their proliferation.

In the mortality-I and mortality-II experiments different proportions of trophonts and plasmodia were used (for definitions see below). The mortality-I experiment consisted of a control group of uninfected crabs (n=22) injected individually with 100 µL

of hemolymph from an uninfected donor crab and an experimental group (n=20) injected individually with 100 µL of infected hemolymph from a donor crab containing an estimated 1.3×10^6 trophonts/mL (1.3×10^5 trophonts per crab).

The mortality-II experiment consisted of a control group (n=8) injected individually with 100 µL of physiological saline buffer (modified from Appleton and Vickerman, 1998; NaCl, 19.31 g/L; KCl 0.65 g/L; $CaCl_2 \cdot 2H_2O$ 1.38 g/L; $MgSO_4 \cdot 7H_2O$ 1.73 g/L; Na_2SO_4 0.38 g/L; HEPES 0.82 g/L;) adjusted to pH 7.8, with added glucose (1.0 mg/mL) and two experimental treatments (high dose=1.0×10^5 parasites/crab; low dose=1.0×10^3 parasites per/crab, n=10, 10 respectively). To prepare the inoculum for the experimental treatments, 2.0 mL of infected hemolymph were drawn from a donor crab infected with 6.15×10^7 parasites/mL (comprising 97% plasmodia; 3% trophonts). The infected hemolymph was diluted 1:1 with buffer, centrifuged at 4000 rpm for 10 minutes, the supernatant was decanted, and the cells were resuspended in buffer. The cells were then adjusted to 1.0×10^7 parasites/mL, centrifuged through two more washes, and serially diluted to attain densities of 1.0×10^6 parasites/mL and 1.0×10^4 parasites/mL (for inoculum of 100 µL, 1.0×10^5 parasites/crab and 1.0×10^3 parasites/crab, respectively).

In both experiments, crabs were monitored daily for mortalities. Deaths within the first nine days of each experiment were excluded because of handling stress arising from infrequent, bacterial infections (e.g. Johnson, 1976). None of the crabs in the experiments were infected with amoebae, microsporans, or overt bacterial infections (but see Welsh and Sizemore, 1985 for background levels of *Vibrio* spp. in hemolymph of *C. sapidus*). Ten crabs from each treatment in the mortality-I experiment, and all of the crabs in the mortality-II experiment were bled approximately weekly to assess infection status. In the mortality-I experiment, the same ten crabs were bled approximately weekly until they died; other crabs from within the experiment were added as replacements.

Crab hemolymph was taken by using a tuberculin syringe (1 mL) with a 25.5-ga. needle from the arthrodial membrane at the juncture of the basis and the ischium of the 5th pereopod (swimming leg). Ethanol (70%) was used to sterilize the site of inoculation and blood letting. Total and differential counts of host hemocytes and estimates of parasite density were obtained from individual crabs with a hemocytometer (Neubauer improved, Bright Line, two counts per crab) with phase contrast microscopy at 400×. Host hemocytes were identified as granulocytes, semigranulocytes (intermediate cells with relatively few granules, Bodammer, 1978; Johnson, 1980) and hyalinocytes (cell types defined in Söderhäll and Cerenius, 1992). Hemocyte and parasite densities higher than 1.0×10^7 cells/mL were diluted 1:5 with buffer and recounted to provide better estimates of cell density. For comparative purposes, total hemocyte densities and differential counts from naturally infected male and female crabs were also obtained.

Parasites were easily distinguished from host cells by using phase contrast microscopy (Fig. 1): uninucleate trophonts (9–15 µm) possessed few small, refractile vacuoles and were rounded or amoeboid, without filopodia; multinucleate plasmodia (20–100 µm) were slender, vermiform, and motile. The density of infection refers to the number of parasites per mL of hemolymph. Total hemocyte density refers to the number of hemocytes per mL of hemolymph. Mean intensity refers to the mean number of parasites per quantity of *infected* host tissue (Margolis et al., 1982).

Permanent preparations of hemolymph were processed and stained as described in Messick (1994). Briefly, acid-cleaned, poly-l-lysine-coated microslides were smeared with fresh hemolymph, allowed to stand for 2–3 minutes, and fixed in Bouin's fixative. The smears were processed through a routine Harris hematoxylin and eosin-Y procedure (Humason, 1979, p. 123 without acid destain).

The early infection experiment consisted of a control group (n=5 crabs) injected individually with 100 µL of hemolymph from an uninfected donor crab and an experimental group (n=20) injected with 100 µL of hemolymph from a donor crab containing an estimated 4.1×10^6 parasites/ml (4.1×10^5 parasites per crab; comprising 79% plasmodia, 21% trophonts). Three days prior to infection, cell counts were conducted on all crabs to serve as a benchmark (presample) for before-after comparisons. On days 3, 5, and 7 after inoculation, five infected crabs were bled and dissected. Differential cell counts were conducted and tissue samples taken for histological analysis. Tissue samples were processed through a routine hematoxylin and eosin procedure and included muscle, hepatopancreas, heart, and, in some cases, foregut. The control crabs were bled and tissue samples taken 10 days after injection.

For statistical analyses, the proportional hazards model with the Weibull distribution was used to examine survival data and associated variables (Cox and Oakes, 1984). The Tarone-Ware log-rank test was used to examine differences between survival curves (Wilkinson, 1997). ANOVA was used to analyze relationships in hemocyte densities and proportion of cell type (cell type density divided by total hemocyte density) between inoculated and uninfected crabs. Simi-

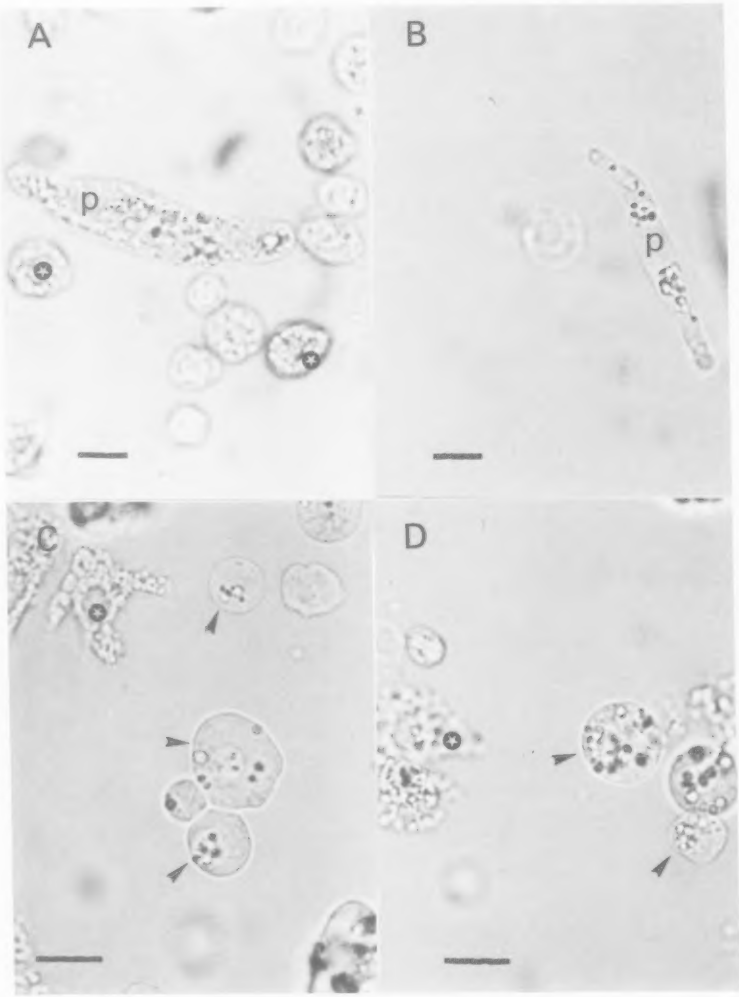


Figure 1

Hematodinium perezi from the blue crab, *Callinectes sapidus*. (**A and B**) Vermiform plasmodia (p) in hemolymph (granulocytes, star). Bar = 10 µm. (**C**) Amoeboid trophonts (arrows) with few refractile granules. Bar = 10 µm. (**D**) Round trophonts (arrows) with many refractile granules. Bar = 10 µm.

lar densities and proportions of cell types were noted in hematology and survival between the mortality-I and mortality-II experiments; hence, data were combined *a posteriori* for the analyses. Where similar trends were noted between statistics for injection dosage (10^3 vs. 10^5), data were also combined for the analysis (i.e. survivorship, hematology). SYSTAT (Wilkinson, 1997) and SAS (SAS, 1988) were used for the analyses. A probability level of $P < 0.05$ was accepted as significant.

Results

Inoculated crabs that became infected with *Hematodinium perezi* began dying two weeks after inoculation (Fig. 2). Mortalities peaked at three weeks after injection and continued to accumulate from weeks 3 through 5. The mortality rate of the infected crabs was 86%, whereas less than 20% of the controls died. Crab mortalities were similar over the time course of infection between mortality-I (infected

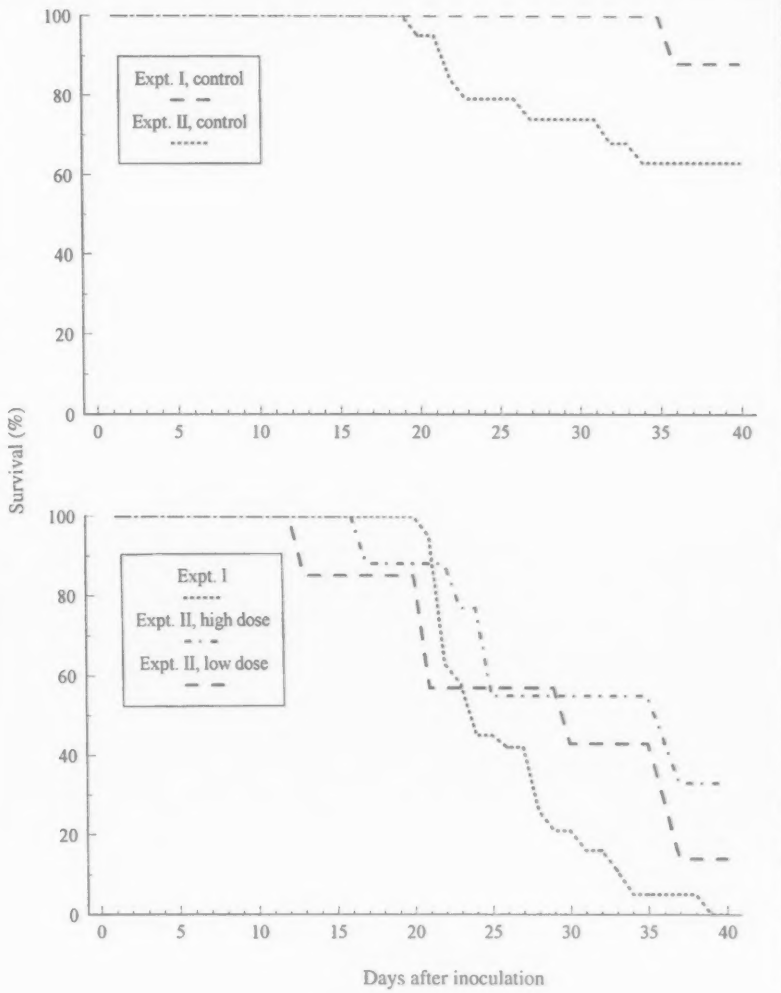
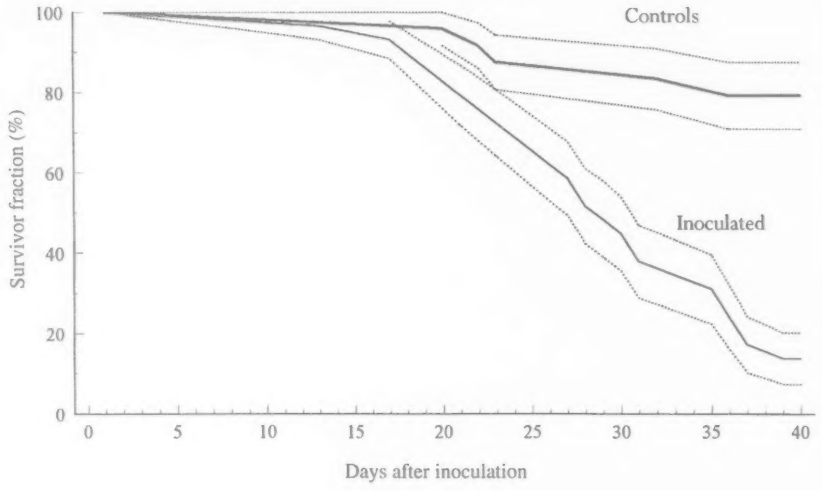


Figure 2

Survivorship curves for uninfected crabs and crabs infected with *H. perezi* (high dose=10^5 parasites/crab, low=10^3 parasites/crab). Sample sizes were 22 and 8 uninfected controls; 20 (mortality-I experiment), 10 and 10 (high and low dose, mortality-II experiment) respectively.

hemolymph, uninucleate trophonts) and mortality-II (buffer-washed parasites, vermiform plasmodia) experiments (Tarone-Ware, χ^2=1.21 with 1 df, P=0.27), even between different initial doses of the parasite (Fig. 2; Tarone-Ware, χ^2=0.74 with 1 df , P= 0.39). Uninfected crabs (controls) experienced significantly fewer mortalities than did infected hosts (Fig. 3; Tarone-Ware, χ^2=, 19.27 with 1 df , P<0.001). The controls for the mortality-II experiment did, however, exhibit background mortalities (Fig. 2); but the mortality rate was not significantly different from controls in the mortality-1 experiment (Tarone-Ware, χ^2=0.65 with 1 df, P= 0.42). None of the control crabs developed infections with *H. perezi*. Because mortalities within treatments were similar between experiments, data were grouped for further analysis.

The median time to death for infected crabs was 30.3 ±1.5 (SE) days. Because the controls exhibited few mortalities, the median time to death for the uninfected controls could not be calculated. Infected crabs had a significantly higher mortality rate, seven to eight times greater than that of the uninfected con-



VARIABLE	ESTIMATE	SE	χ^2	P
Intercept	2.933	0.106	763.33	0.0001
Uninfected	1.055	0.287	13.50	0.0002
Injected	0	0		
Scale	0.517	0.083		

Figure 3

Survival function (Kaplan-Meier estimation) for uninfected crabs and crabs infected with *H. perezi*. Dashed lines are upper and lower standard errors. Data include all crabs from mortality-I and mortality-II experiments. Sample sizes are given in Figure 1.

trols (Fig. 3; proportional hazards, χ^2=13.50, P<0.001; relative risk=$e^{1.055/0.5174}$). Hemocyte and parasite density were jointly analyzed as covariates in the proportional hazards model. For injected crabs, the decline in ln(total hemocyte density) was significantly associated with mortality (ln *day of death* = 0.875 + 0.145 ln *total hemocyte density* – 0.017 ln *Parasite density* + 0.409 W; χ^2=4.47 with 1 df, P<0.05). Hemocyte density (untransformed), and parasite density (ln, and untransformed) were not associated with mortality (χ^2, P=0.07, 0.61, and 0.47, respectively); thus, decreases in hemocyte density (ln), not parasite density, were associated with imminent death.

Direct observations from crabs used to maintain infections and experimental results indicated that the parasite was detectable in the hemolymph approximately two weeks after injection (Fig. 4). Although the parasite could be detected as early as one week after inoculation, detectability (the percentage of infected crabs exhibiting detectable parasites in the hemolymph) was relatively low (30–35%) after 14 to 18 days, reaching 80–85% after 26 to 32 days, and 100% after 35 days. (Detectability was based solely on inoculated animals that developed infections. The four crabs from the mortality-II experiment that did not present infections, hereafter referred to as "immune" crabs, were excluded from the analysis of detectability.) Proliferation and growth of the parasite followed a similar pattern as detectability, and the two variables were clearly related (Table 1; Fig. 4).

Growth of the parasite showed a marked increase in the mean density of vermiform plasmodia over days 18 to 26 (Table 1). The mean density of trophonts increased markedly over days 32 to 35. Note, however, that to avoid mortalities from other causes (e.g. secondary infections), sampling could not be done on a daily basis.

Plasmodia were found within the hearts of 93% (n=14/15) of the injected crabs in the early infection experiment. Plasmodia were found in 4 of 5 crabs as early as day 3 (Table 2). Uninucleate trophonts were observed in the heart on and after day 7. Rel-

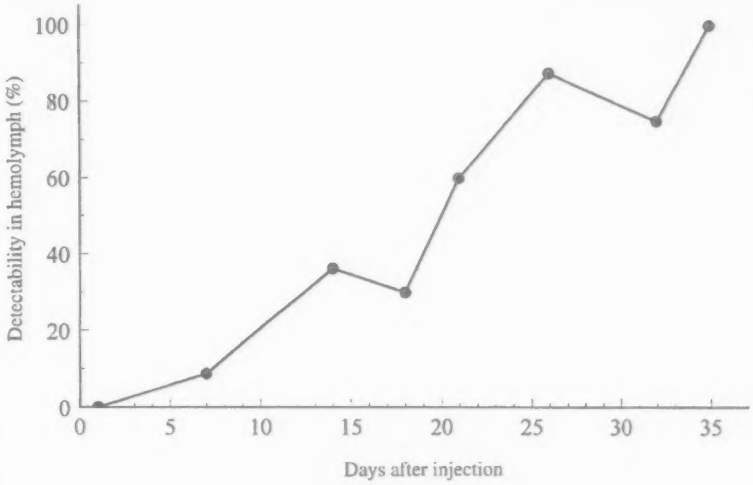


Figure 4

Detectability of parasites in hemolymph of infected blue crabs over the course of infection. Data were combined from mortality-I and mortality-II experiments and include only infected crabs. Samples sizes were 21, 11, 10, 10, 16, 4, and 4 crabs on days 7, 14, 18, 21, 26, 32, and 35, respectively.

Table 1

Parasite intensity ($\times 10^5$ parasites/mL per infected host) in the hemolymph in relation to days after inoculation. Counts combined for mortality-I and mortality-II experiments. N_{plas} = crabs with plasmodia, N_{troph} = crabs with trophonts, $N_{infected}$ = infected crabs exhibiting parasites. See Table 3 for sample sizes for hemocytometry.

		Plasmodia	log(plasmodia)		Trophont	log(trophont)	
Days	N_{plas}	Mean ±SE	Mean ±SE	N_{troph}	Mean ±SE	Mean ±SE	$N_{infected}$
7	1	0.50 ±0.00	4.70 ±0.00	1	0.25 ±0.00	4.40 ±0.00	1
14	4	1.44 ±0.53	5.09 ±0.16	3	1.25 ±0.29	5.06 ±0.11	4
18	1	1.25 ±0.00	5.10 ±0.00	2	1.00 ±0.25	4.99 ±0.11	3
21	5	7.70 ±4.61	5.61 ±0.24	5	8.65 ±4.17	5.87 ±0.18	5
26	11	14.98 ±6.07	5.85 ±0.17	12	8.19 ±3.86	5.40 ±0.22	14
32	2	8.38 ±1.63	5.92 ±0.09	3	4.25 ±1.32	5.59 ±0.14	3
35	4	7.88 ±3.89	5.69 ±0.25	4	49.81 ±36.82	6.35 ±0.31	4

atively more parasites were observed in the heart tissue over time (Table 2); but no effort was made to standardize area in the histological preparations. Growth of the parasite was rapid in the heart. The dosage in the early infection experiment was, however, four times higher than that in the mortality-I and mortality-II experiments; thus, results between experiments were not directly comparable.

Sporulation from the trophont stage to the dinospore stage was observed only in crabs that were used to maintain infections. Parasites in one crab sporulated at least twice and each event lasted less than 4 d. Parasite density was extraordinarily high (1.6×10^8 dinospores/mL) during sporulation, and dropped to moderate levels (3.3×10^6 trophonts/mL) thereafter. Dinospores were observed five times over the course of 26 d, beginning 43 d after injection. Additionally, some crabs injected with only the trophont (vegetative) stage were observed with plasmodia after 3 to 4 weeks of infection.

Hemograms of infected crabs were significantly different from those of uninfected controls (Tables

3 and 4, Fig. 5). Total hemocyte density was significantly depressed in infected crabs (Fig. 5A; 2-way ANOVA by group and day, F=5.03, P<0.001). Total hemocyte density was not significantly different between crabs inoculated with different initial doses (2-way ANOVA, 10^3 vs. 10^5 parasites per crab and day, F=3.19, df=1, 64, P=0.079). Crabs that were injected and did not acquire the infection ("immune" hosts) did not have significant decreases in hemocyte densities (Table 3, Fig. 5; 2-way ANOVA, F=1.46, df = 13, 105, P=0.145). In the early infection experiment, the decrease in total hemocyte densities occurred within three days of inoculation (Table 5).

In addition to a decrease in cell density, the proportions of different host cell types (density of cell type divided by total hemocyte density) in infected crabs shifted to those with proportionally more granulocytes than hyalinocytes (Table 6, Fig. 5, B and D) (2-way ANOVA, F=1.83; df=20, 149, P<0.05). Significant shifts in the population of semigranulocytes were also noted (F=2.51, df =20, 149, P<0.001).

Table 2

Relative intensity of plasmodia in histological preparations of heart sections of mature, nonovigerous female blue crabs from the early infection experiment. Mean intensities represent direct counts of plasmodia and are not standardized by tissue area.

Day	$N_{Infected}/N_{Injected}$	Mean intensity (±SD)	Range
Control	0/4	0.0 ±0.0	—
3	4/5	3.6 ±3.9	1–10
5	5/5	12.0 ±11.0	1–26
7	5/5	55.8 ±26.1	15–74

Table 3

Total mortality-hemocyte densities ($\times 10^6$ hemocytes/mL) in relation to days after inoculation. Hemocyte counts were combined from mortality-I and mortality-II experiments. (-- = not done, no infected crabs survived to day 40).

	Uninfected control crabs		Inoculated, infected crabs		Inoculated, immune crabs	
		Hemocyte density		Hemocyte density		Hemocyte density
Days	N	Mean ±SE	N	Mean ±SE	N	Mean ±SE
7	18	29.17 ±3.09	22	14.39 ±2.05	4	22.99 ±2.32
14	8	32.81 ±3.01	11	16.10 ±3.19	4	33.25 ±2.76
18	9	32.28 ±6.72	10	12.24 ±1.51	--	--
21	8	23.83 ±2.20	10	17.68 ±4.39	4	32.72 ±1.04
26	18	26.65 ±2.15	16	7.64 ±1.49	4	26.29 ±8.00
32	12	20.97 ±3.41	4	4.21 ±2.04	--	--
35	8	23.37 ±4.48	4	10.86 ±6.73	4	23.61 ±11.72
40	10	20.35 ±2.12	--	All dead	4	22.53 ±5.69

Table 4

Total and differential hemocyte densities (mean ±SE; $\times 10^6$ hemocytes/mL) in naturally infected male and female blue crabs in relation to severity of infection (light < 4.0×10^5 parasites/mL; moderate = 4.0×10^5 to 2.0×10^6 parasites/mL; heavy > 2.0×10^6 parasites/mL; -- = not done).

Severity	n	Hemocyte density	Granulocyte density	Semigranulocyte density	Hyalinocyte density
Mature males					
Light	4	16.16 ±2.67	5.46 ±1.41	7.69 ±2.07	3.02 ±0.61
Moderate	6	7.46 ±1.74	1.92 ±0.63	4.10 ±1.10	1.45 ±0.46
Heavy	16	6.66 ±2.53	1.34 ±0.53	3.39 ±1.35	1.93 ±0.76
Mature females					
Light	--	--	--	--	--
Moderate	5	22.41 ±11.25	9.32 ±5.15	5.44 ±2.85	7.65 ±3.66
Heavy	5	14.37 ±7.37	5.98 ±3.09	4.01 ±1.63	4.39 ±2.89

"Immune" crabs exhibited a fluctuation in cell types with significantly higher proportions of granulocytes to semigranulocytes during the first five weeks after inoculation (F=4.35, df =5, 18, P<0.01). By day 40, the hemograms of "immune" hosts were virtually identical to those of the uninfected controls (Table 7, Fig. 5, C and D).

In the early infection experiment, hemocyte populations shifted within the first three days of infection (Tables 5 and 6; ANOVA, log hemocytes, F=9.16; df =3, 31, P<0.01); the proportion of granulocytes in infected crabs increased significantly compared with the proportion of semigranulocytes (ANOVA, F=4.39, P<0.05). Uninfected crabs exhibited minor fluctuations in the proportion of granulocytes to that of hyalinocytes but the proportions were similar to those observed in the mortality-I and mortality-II experiments (Tables 6 and 7).

Discussion

In laboratory experiments, *Hematodinium perezi* caused significant mortality to infected mature, nonovigerous blue crabs. Infections were not always fatal (four crabs survived inoculation without developing infections), but the overall mortality to laboratory-inoculated crabs was high at 86% over 40 days. The proportional hazards model indicated that infected crabs were seven to eight times more likely to die than uninfected crabs. Infections in Tanner crab, *Chionoecetes bairdi*, and Norway lobster, *Nephrops norvegicus*, are frequently fatal to the host (Meyers et al., 1987; Field et al., 1992). The mortality of naturally infected Tanner crabs held in aquaria for 97 days was 67% (n=11) and hosts survived from 20 to 158 days in the laboratory. Uninfected Tanner crabs experienced no mortality during the course of the experiment (Meyers et al., 1987). Naturally infected Norway lobsters suffered mortality rates of 86% to 100% over 27 d and 75 d, respectively, and had mortality rates 2–4 times higher than uninfected lobsters, and most of the deaths occurred early in the course of the experiment (Field et al., 1992).

During epizootics, juvenile blue crabs have a higher prevalence of *H. perezi* than do mature hosts (Messick, 1994). Male blue crabs have a prevalence of infection similar to that for females along the

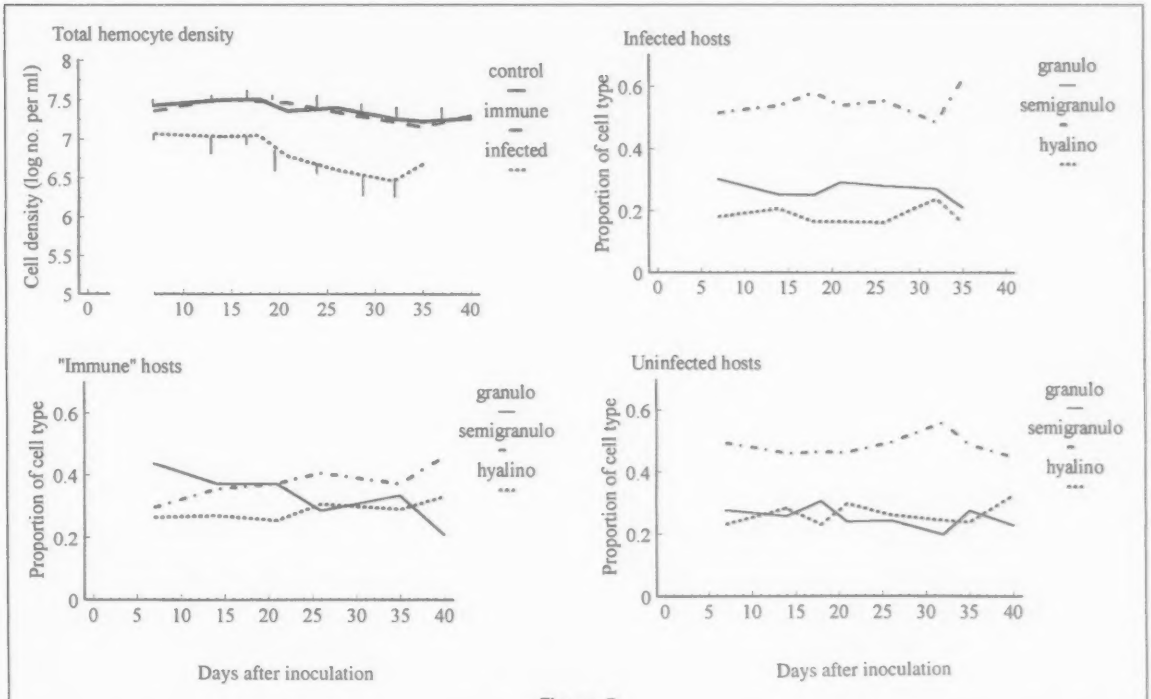


Figure 5

Total hemocyte densities and proportions of host cell types in uninfected, infected and "immune" crabs. Data combined from mortality-I and mortality-II experiments. Bars = SE. Standard errors (not shown) for proportion of host cell types were low (0.02–0.05). "Immune" crabs were survivors from mortality-II experiment that never developed infections. Sample sizes given in Table 3.

Table 5

Differential densities of hemocytes ($\times 10^6$ hemocytes/ml) in relation to days after inoculation for crabs in the early infection experiment. Sample sizes are given in Table 2.

	Granulocyte density	Semigranulocyte density	Hyalinocyte density
Days	Mean ±SE	Mean ±SE	Mean ±SE
Uninfected control crabs			
Presample	3.72 ±0.31	7.66 ±0.50	4.53 ±0.79
10	7.48 ±1.60	11.20 ±1.94	5.30 ±1.47
Inoculated, infected crabs			
Presample	6.88 ±0.92	11.43 ±1.00	4.97 ±0.40
3	4.95 ±0.40	4.36 ±0.31	2.80 ±0.15
5	2.51 ±0.91	5.40 ±1.60	1.49 ±0.51
7	2.93 ±0.68	6.92 ±0.80	1.56 ±0.31

Delmarva Peninsula (Messick, 1994; Messick and Shields[4]) and show significantly greater changes than females in certain blood parameters.[5] Infected blue crabs apparently die before acquiring the bitter flavor found in infected Tanner and snow crabs.

Survival analysis indicated that parasite density was not associated with mortality. Similarly, survival time of Norway lobsters did not show a significant relationship with severity of infection, but host mortality did increase with the progression of the disease (Field et al., 1992). In blue crabs, absolute declines in ln(total hemocyte density) were associated with host mortality. Hence, the cellular defensive response of the host appeared seriously compromised by infection. Anecdotal evidence from hosts used to maintain the parasite suggests that infections established with plasmodia are more pathogenic than those established with trophonts; this may explain the similar mortality curves for the high and low doses of plasmodia in the mortality-II experiment. Observations on naturally and experimentally infected crabs indicate three possible outcomes to the disease:

1 Crabs with acute infections, such as those reported here, show rapid mortalities, typically dying within 40 d. Acute infections rarely lead to heavy infections (10^{7+} parasites/mL), and may not lead to the development of dinospores.

Table 6

Total hemocyte densities ($\times 10^6$ hemocytes/mL) in relation to days after inoculation (-- = not done) in the early infection experiment.

	Uninfected control crabs		Inoculated, infected crabs	
		Hemocyte density		Hemocyte density
Days	*n*	Mean ±SE	*n*	Mean ±SE
Presample	5	15.91 ±1.25	20	23.28 ±2.06
3	--	--	5	12.11 ±0.50
5	--	--	5	9.39 ±2.94
7	--	--	5	11.40 ±0.96
10	5	23.98 ±4.87	--	--

2 Crabs with chronic infections (observed in very cases, n=4) endure the acute stage, survive for longer periods (up to 90 days), and develop infections that lead to massive numbers of dinospores (Fig. 6).
3 Some crabs successfully resist the parasite or are refractory to the infection. Preliminary experiments (not shown) suggest that resistant crabs (n=10) may become refractory to further inoculations with *H. perezi*.

Blue crab catches fluctuate yearly in Chesapeake Bay but causes for these fluctuations are not well understood. Since salinity appears to limit the distribution of *H. perezi* (Newman and Johnson, 1975), the dinoflagellate could feasibly infect and cause significant mortalities to juvenile and adult crabs

[4] Messick, G. and J. D. Shields. 1999. The epizootiology of the parasitic dinoflagellate *Hematodinium perezi* in the blue crab, *Callinectes sapidus*. Oxford Cooperative Laboratory, 904 S. Morris St., Oxford, MD 21654. Unpubl. data.

[5] Shields, J. D. 1999. Mortality and pathophysiology studies of blue crabs infected with the parasitic dinoflagellate *Hematodinium perezi*. Saltonstall-Kennedy Program. NOAA/National Marine Fisheries Service. Final Report.

Table 7

Differential densities of hemocytes ($\times 10^6$ hemocytes/mL) in relation to days after inoculation. Hemocyte counts were combined from mortality-I and mortality-II experiments (-- = no data, no infected crabs survived to day 40). Sample sizes are given in Table 3.

	Granulocyte density	Semigranulocyte density	Hyalinocyte density
Days	Mean ±SE	Mean ±SE	Mean ±SE
Uninfected control crabs			
7	8.13 ±0.97	13.88 ±1.28	7.16 ±1.10
14	8.34 ±1.17	15.13 ±1.66	9.34 ±0.94
18	8.99 ±1.42	15.37 ±3.33	7.83 ±2.18
21	5.88 ±0.93	10.86 ±0.93	7.09 ±0.91
26	6.56 ±0.73	13.06 ±1.04	7.04 ±0.76
32	4.10 ±0.81	12.14 ±2.21	4.73 ±0.53
35	5.64 ±1.15	11.67 ±2.34	6.05 ±1.37
40	4.66 ±0.61	9.29 ±1.13	6.39 ±0.68
Inoculated, infected crabs			
7	5.28 ±1.04	6.18 ±0.71	2.92 ±0.56
14	5.59 ±1.34	6.90 ±1.26	3.62 ±0.94
18	3.29 ±0.67	6.76 ±0.65	2.20 ±0.47
21	4.77 ±1.23	8.37 ±1.75	4.60 ±1.54
26	2.10 ±0.45	4.25 ±0.83	1.28 ±0.36
32	1.11 ±0.57	2.19 ±1.14	0.90 ±0.41
35	3.11 ±2.13	6.11 ±3.58	1.64 ±1.06
40	--	--	--
Inoculated, immune crabs			
7	9.73 ±0.65	7.08 ±1.58	6.19 ±0.93
14	12.31 ±0.83	11.91 ±1.34	9.03 ±1.00
18	--	--	--
21	11.34 ±2.51	11.94 ±3.44	9.44 ±4.75
26	8.16 ±3.73	10.00 ±2.55	8.14 ±2.80
32	--	--	--
35	7.98 ±4.03	8.03 ±3.89	7.61 ±3.82
40	4.59 ±1.35	10.00 ±2.30	7.94 ±2.71

where salinities are greater than 11‰; i.e. much of the mainstem of Chesapeake Bay. Current models for blue crab populations in Chesapeake Bay are based on population assessments from various surveys (Lipcius and Van Engel, 1990; Abbe and Stagg, 1996; Rugolo et al., 1998). These models project crab abundance for the fishery as a whole but do not separate the larger, low-salinity "bayside" fishery from the smaller, high-salinity "seaside" fishery where mortalities due to *H. perezi* occur. Such projections include estimates of natural mortalities but do not account for the potential epizootics and resulting mortalities caused by *H. perezi*. Differential models of exploitation by region may be warranted, especially during or immediately following epizootics. Disease estimates must, however, account for the variation in the prevalence of detection because the prevalence in field samples may be significantly underreported (see Fig. 3).

The life cycle of *H. perezi* from *C. sapidus* has not been fully documented. Several morphological and life history differences, however, distinctly separate *Hematodinium* sp. ex *N. norvegicus* (Appleton and Vickerman, 1998) from *H. perezi* ex *C. sapidus*. For example, the syncytial and network forms of *Hematodinium* sp. ex *N. norvegicus* (Field and Appleton, 1995) have not been observed with *H. perezi* (Messick, 1994; present study); nor do the plasmodia (cf. filamentous trophonts of Appleton and Vickerman, 1998) of *H. perezi* develop as "gorgonlocks"; rather, they undergo budding to produce additional plasmodia, and schizogony (cf. segmentation in malaria life cycles) to produce uninucleate trophonts (senior author, unpubl. data). The trophonts then undergo further fission. Such differences may warrant generic separation between the two parasites.

Hematodinium perezi was successfully transmitted to blue crabs by injection. Transmission experiments

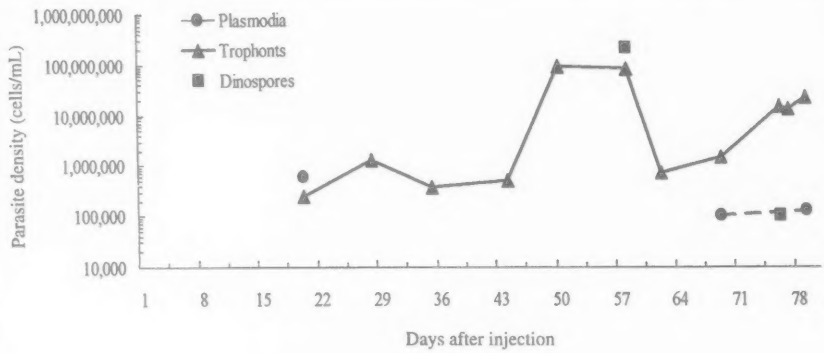


Figure 6

Crab no. 3977 presenting a chronic, experimentally induced infection of *H. perezi* (plasmodia, circles; trophonts, triangles; dinospores, boxes). The crab died after 80 days.

with the parasite in Tanner crabs and Australian sand crabs (*Portunus pelagicus*) have been partially successful. Parasites from primary cell culture (with sterile hemolymph) were successful in establishing infections in Tanner crabs, but inoculation with vegetative stages did not produce infections (Meyers et al., 1987). Injections of trophonts (vegetative stages) were successful in producing infections in the sand crab, but other stages were not investigated (Hudson and Shields, 1994). Infection experiments with Norway lobster have not been reported. Transmission with cultured dinospores has yet to be achieved (Appleton and Vickerman, 1998). Sporulation is a rapid event with *H. perezi*, presumably occurring over several hours instead of several days or weeks as reported for *Hematodinium* sp. from Tanner crabs (Meyers et al., 1987, 1990). At lower temperatures and salinities, *H. perezi* apparently ceases to grow or slows its proliferation in naturally infected blue crabs.[6]

Densities of circulating hemocytes declined rapidly in infected blue crabs. The decline occurred within the first three days and progressed to a 48% decrease in total hemocyte densities within the first week of infection. After three weeks, absolute declines of up to 80% were noted for total hemocyte densities. The loss of cells was evident early in the infection even though the parasites were not detectable in hemolymph. Declines in hemocyte densities have been reported for starved lobsters (33–60% loss) (Stewart et al., 1967), *Aeromonas*-infected lobsters (80–84% loss) (Stewart et al., 1983), *Fusarium*-infected brown shrimp, *Penaeus californiensis* (approximately 88% loss) (Hose et al., 1984), and *Vibrio*-infected *Cancer irroratus* (95% loss) (Newman and Feng, 1982). Reductions in hemocytes were noted for Norway lobster, *N. norvegicus*, infected with *Hematodinium* sp. (Field and Appleton, 1995), and for blue crabs, *C. sapidus*, infected with *Paramoeba perniciosa* (Sawyer et al., 1970), but the degree of loss, and differential counts were not quantified. Declines in hemocyte counts occur quickly in crayfish, *Pacifastacus leniusculus*, (10 min) and are associated with loss of resistance to *Aphanomyces* infections; the declines are dependent upon the stimuli (yeast vs. zymosan vs. buffers), and are evident over the course of several days (Perrson et al., 1987).

Crustacean cell types probably represent maturation of a single lineage (e.g. Bodammer, 1978; Bachau, 1981; Hose et al., 1990). Hyalinocytes represent younger cells that become semigranulocytes (intermediate hemocytes), then granulocytes. Infected crabs exhibited marked shifts in subpopulations of different hemocyte stages (cell types). Because there was an absolute decline in the total number of circulating hemocytes and relative declines in hyalinocytes and semigranulocytes, we suggest that cell death and differential sequestration occur in response to the disease. General declines in hemocyte density in *N. norvegicus* infected with *Hematodinium* sp. may occur from sequestration, other defense reactions, and hydrostatic effects of heavy infections or clogging of hemal sinuses (Field and Appleton, 1995). In our study, the rapid decline in total hemocyte density (within three to seven days) argues against hydrostatic effects and clogged sinuses. The shift towards proportionally more granulocytes than hyalinocytes may result from mobilization of tissue-dwelling reserves, differential cell death (Mix and

[6] Messick, G. 1998. Oxford Cooperative Laboratory, 904 S. Morris St., Oxford, MD 21654. Personal. commun.

Sparks, 1980), changes in mitotic stimuli of hemopoetic tissue (Hose et al., 1984), or sequestration of specific cell types in defense of the infection (e.g. nodule formation, Johnson, 1976, 1977). Hyalinocytes and semigranulocytes are the major phagocytic hemocytes in crustaceans (Bachau, 1981; Hose et al., 1990). Such hemocytes form nodules in bacterial, amoebic, and *Hematodinium* infections in blue crabs, *Hematodinium* infections in *N. norvegicus*, and gaffkemia infections in *Homarus americanus* (Johnson, 1976, 1977; Johnson et al., 1981; Messick, 1994; Field and Appleton, 1995) are thus removed from circulation, and may account, in part, for the observed declines. In fungal infections of crayfish, *P. leniusculus*, hemocytic nodules do not dissociate in the presence of zymosan, a yeast derivative, and may last several days (Perrson et al., 1987). In *Aeromonas*-infected lobsters, the hyalinocytes increase in proportion to the other cell types, presumably by increased mitotic activity in hemopoeitic centers, but there is a significant decline in hemocytes after five days of infection (Stewart et al., 1983).

Lastly, several blue crabs (n=4) successfully fought off the infection. These "immune" crabs exhibited significant sustained levels of granulocytes, did not suffer hemocytopenia, their hemolymph clotted normally, and they did not exhibit gross changes in morbidity. Histological preparations of heart, hepatopancreas, muscle, and hemopoeitic tissues were negative for latent infections in the "immune" animals. The relative increase in semigranulocytes and sustained densities of hyalinocytes over time (Fig. 5) suggests an increase in mitotic activity in hemopoetic tissue in response to the infection. This increase may not be sufficient to counter the parasite in susceptible hosts. In *N. norvegicus* infected with *Hematodinium* sp., the hemopoeitic tissues show marked increases in activity. Although changes in host cell densities were not quantified, apparent stem cells were present in the active nodes (Tables 1 and 2 in Field and Appleton, 1995). The role of the granulocytes in the defense against *Hematodinium* infections and the underlying mechanisms leading to refractory hosts warrant further study.

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Age determination and growth of Hudson River Atlantic sturgeon, *Acipenser oxyrinchus**

Jill T. Stevenson
David H. Secor
Center for Environmental Science
Chesapeake Biological Laboratory
University of Maryland
P.O. Box 38
Solomons, Maryland 20688-0038
Present address (for J. Stevenson): Highly Migratory Species Management Division, F/SF1
National Marine Fisheries Service
1315 East-West Highway
Silver Spring, MD 20910
Email address (for J. Stevenson): jill.stevenson@noaa.gov

Abstract.—Atlantic sturgeon (*Acipenser oxyrinchus*) are a large anadromous fish which is especially vulnerable to overharvesting owing to its late age of maturity and low rate of reproduction. Age determination methods and growth rates are poorly described for this species. Pectoral-fin spine sections and sagittal otolith sections were examined to determine whether one of these structures would be useful in estimating the age and growth of Atlantic sturgeon. Otoliths have been difficult to collect, process, and interpret. Interpretation of annuli in sectioned pectoral spines has proven to be an unbiased method for aging juvenile and adult Hudson River Atlantic sturgeon. Marginal increment analysis has indicated an annual cycle of annulus deposition. Microchemical analysis with an electron microprobe of the periphery of fin spines has shown seasonal patterns of calcium and phosphorus concentrations related to the translucent and opaque zones of the annuli. Formation of yearly annuli was verified in 4-year-old laboratory-reared sturgeon. Von Bertalanffy growth models (based upon fin-spine interpretations) were fitted for the Hudson River population. Models predicted a more rapid growth rate for males than for females (females: K=0.07, L_∞=251 cm; males: K=0.25, L_∞=180 cm). Females, however, attained a greater maximum age (42 yr) and size (TL=277 cm). We believe that exploitation has had a large but unquantifiable bias on growth estimates for male and female Atlantic sturgeon. As the Hudson River population recovers, age structure and growth rate estimates should be refined to predict population recovery rates more accurately in the absence of a directed fishery.

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Atlantic sturgeon (*Acipenser oxyrinchus*) are a large anadromous fish that ranges the East Coast of North America and spawns in rivers from Florida to Canada. Population levels throughout the range of the species declined appreciably in the late 19th century owing to increased harvest of sturgeon for caviar following the Civil War (Murawski and Pacheco, 1977; Secor and Waldman, in press). Overfishing and deterioration of habitat, predominantly the blockage of spawning runs, have contributed to the extirpation of several Atlantic sturgeon populations (Taub, 1990; Waldman and Wirgin[1]). The life history strategy of the anadromous Atlantic sturgeon indicates that age structure and vital rates are especially critical to conservation. Atlantic sturgeon exhibit high maximum age, late maturation (females 14–17, males 10–12; Van Eenennaam et al., 1996), and probable low mortality rates; growth, however, is rapid. These traits, as well as low relative fecundity and less-than-annual spawning frequency, make sturgeon especially susceptible to overexploitation (Boreman, 1997). Therefore, models of Atlantic sturgeon population dynamics may be expected to be sensitive to biases in estimated vital rates and reproductive schedules. Atlantic sturgeon growth rates have been estimated in several studies, but results are divergent (Table 1). Poorly validated techniques have been employed to estimate age; and rates of growth, reproduction, and mortality have not been developed sufficiently to support resource management models (Taub, 1990).

Studies of acipenserid age have employed annuli in calcified structures including scutes, pectoral-fin spines, otoliths, operculi, and other skeletal parts (Harkness, 1923; Greeley, 1937; Brennan and Cailliet, 1989, 1991; Guenette et al., 1992; Rien and Beamesderfer, 1994). The term "fin ray," previously used to describe the leading (primary) ray of the pectoral fin supporting element, was revised by Feindeis (1997) because this element becomes fully ensheathed with dermal bone early in ontogeny, and therefore should be termed a spine. Pectoral-fin spine sections have been preferred for aging because annuli in sections can be consistently interpreted, and fin spines are easily col-

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[1] Waldman, J. R., and I. I. Wirgin. 1998. Status and restoration options for Atlantic sturgeon in North America. ICES Council Meeting/T 16.

Table 1

Imprecision in aging studies of various long-lived species, reported as coefficient of variation (CV).

Species	Study	CV	Maximum age observed
Atlantic sturgeon (*Acipenser oxyrinchus*) (fin spines)	This study	4.8	42
White sturgeon (*Acipenser transmontanus*)	Rien and Beamesderfer (1994)	7.8	104
Yellowfin sole (*Pleuronectus asper*)	Kimura and Lyons (1991)	3.2	26
Sablefish	Kimura and Lyons (1991)	12.9	29
Pacific ocean perch (*Sebastes alutus*)	Kimura and Lyons (1991)	4.9	78
Tarpon (*Megalops atlanticus*)	Cyr (1991)	12.0	50

lected and processed without sacrifice of fish. They have been used in the past to age Atlantic sturgeon, but authors have described difficulty in aging older fish (>20 yr; Magnin, 1964; Huff, 1975; Dovel and Berggren, 1983). Aging based upon fin spines has been validated for white sturgeon, *Acipenser transmontanus* (Brennan, 1988; Brennan and Cailliet, 1991) and lake sturgeon, *A. fulvescens* (Rossiter et al., 1995); however, no studies have validated the periodicity of annulus deposition in Atlantic sturgeon.

Otoliths are often preferred for estimating fish age, but rate of otolith annulus formation has not been evaluated in sturgeons. Greeley (1937) enumerated ridges on the external surface of otoliths without examination of an internal section. Subsequent studies have indicated that annuli on an internal section of otoliths do not provide age estimates as precise as those from fin-spine sections (Schneberger and Woodbury, 1944; Brennan and Cailliet, 1991). Otolithic material, however, does not resorb, which is desirable for accurate aging, especially of long-lived fishes.

The objectives of this study were to identify an appropriate calcified structure and develop a precise and unbiased method for determining age of Atlantic sturgeon and to model growth rates of Hudson River Atlantic sturgeon on the basis of juvenile and adult fish collected in 1992–96.

Materials and methods

Adult Atlantic sturgeon were collected during 1992–95 from fishery harvests in the Hudson River and New York Bight, in cooperation with New York State Department of Environmental Conservation and New Jersey Department of Environmental Protection. Fish were collected by using drift and anchored gill nets (25–36 cm stretched mesh) and otter trawls. During the period of collection, a minimum size limit of 152 cm total length (TL) was imposed on the New York fishery. In New Jersey, the minimum size limit of 107 cm TL was replaced with a 152-cm size limit in 1993.

We collected pectoral-fin spines from sturgeon smaller than the minimum commercial size limit (≤149 cm TL) in the Hudson River during 1993–95, using monofilament anchored gill nets (3–13 cm stretched mesh). Because few fish less than 152 cm were collected, other mid-Atlantic Bight regions were sampled. Fin spines were obtained from sturgeon collected from Chesapeake Bay commercial pound nets and gill nets in 1996 (n=11), from National Marine Fisheries Service trawl surveys in the Mid-Atlantic Bight (1994 and 1996; n=4), and from U.S. Fish and Wildlife Service gillnet surveys of the Delaware Bay (n=8). Because the Hudson River stock is the dominant reproducing stock in the Mid-Atlantic area (Waldman et al., 1996), subadults from areas other than the Hudson River were assumed to be predominantly of Hudson River origin.

Removal and preparation of hard parts

Pectoral-fin spines (n=634) were removed at the point of articulation, air-dried, and sectioned less than one centimeter distal to the articulation point. Soft tissue adhering to the fin spines was allowed to decompose through microbial decay. A one-centimeter-wide section of each fin spine was then embedded in a block of Spurr epoxy, sectioned with an Isomet saw (Buehler, Lake Bluff, IL), and mounted on glass slides (see Secor et al., 1991). Some fin spines (64%) were not embedded but were sectioned with a jeweller's saw. All sections were mounted with thermoplastic glue on glass slides and polished with an automated polishing wheel (MINIMET 1000, Buehler, Lake Bluff, IL) with fine grit carborundum paper and a 0.3-µm alumina slurry on a polishing cloth. Final sections were 1–2 mm thick.

Sagittal otoliths were removed from the severed heads of Atlantic sturgeon collected in 1994–95

(n=114). Sagittae were cleaned in 10% bleach, rinsed several times with deionized water, and air-dried. One sagittal otolith from each pair was embedded in Spurr epoxy and sectioned as described in Secor et al. (1991). Owing to their fragility, otolith sections were polished by hand with a variety of fine sandpapers and a 0.03-µm alumina slurry.

Annuli in thin sections of fin spines and otoliths were viewed under reflected light at 15× magnification by two experienced readers. An annulus was defined as a bipartite zone comprising an opaque and a translucent zone (Fig. 1). The first translucent zone was counted as the beginning of the first year of life. In some instances, a secondary fin spine was embedded within the primary fin spine (Feindeis, 1997), in which case care was taken to enumerate annuli only in the primary spine. False annuli were consistently observed in fin-spine sections of older individuals and were excluded from annulus counts. These structures were not continuous around the entire circumference of the section and were thus distinguishable from annuli to be counted (Prince et al., 1985).

Precision and bias

Readers counted annuli without knowing collection date, fish size, or previous age determination. They were trained with the aid of an imaging system that permitted simultaneous observation of annular growth zones. Paired difference tests were used to statistically evaluate bias and precision among readers in a single blind test. The coefficient of variation (CV) was used to measure precision; bias was assessed visually using age-bias plots (Fig. 2). An age-bias plot showed paired estimates of age for the same fish (Campana et al., 1995), with the estimates of reader 2 represented as mean age, and 95% confidence intervals corresponding to each of the age classes estimated by reader 1. For example, if reader 1 estimated five fish to be 15 years old, the age-bias plot indicated the mean age of those five fish as estimated by reader 2. Divergence from the equivalence line, where $Age_{Reader\ 1} = Age_{Reader\ 2}$, indicates a systematic difference between readers. Paired age estimates for either fin-

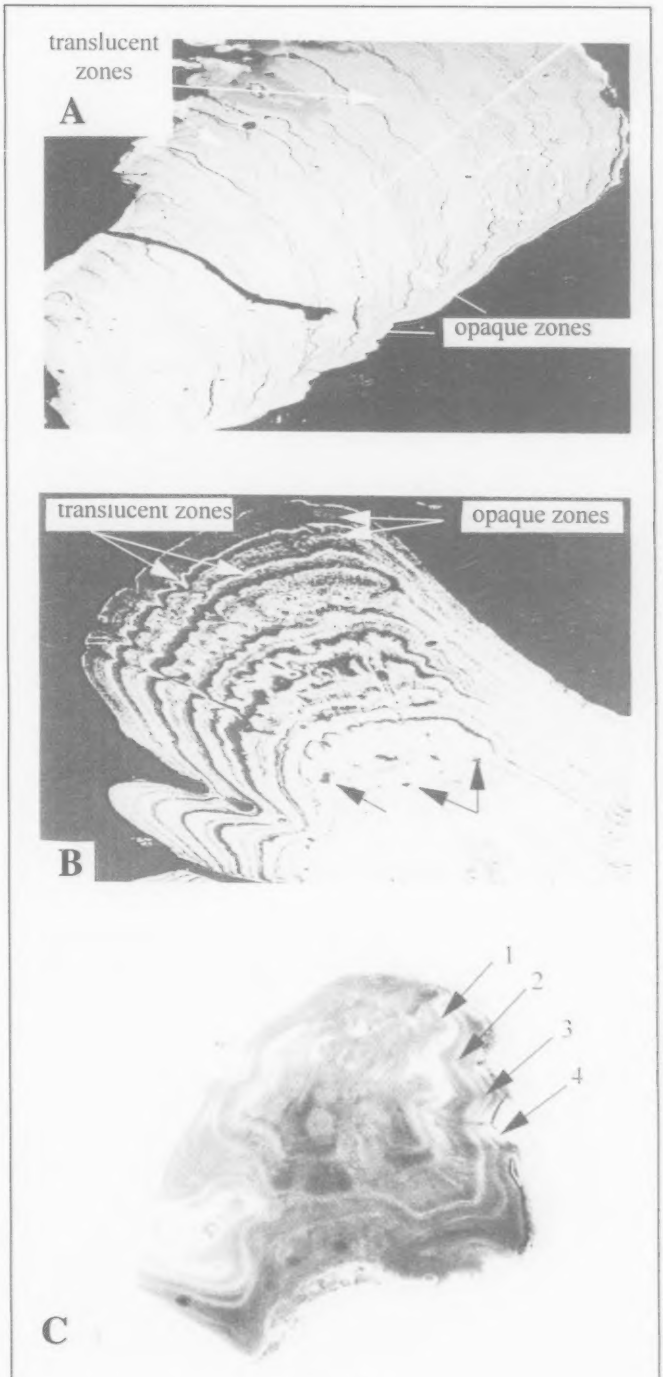

Figure 1

Backscatter electron micrographs show (**A**) a sagittal otolith section; (**B**) a pectoral-fin spine (lower lobe only) from a Hudson River Atlantic sturgeon; and (**C**) a fin-spine section from a hatchery-reared Atlantic sturgeon. White arrows (B) indicate annuli. Organic deposits in the fin-spine section are indicated with dark arrows (B). Pits resulting from chemical microanalysis are visible in the otolith section. Dashed circle in (A) indicates annuli that are difficult to interpret.

spine sections or otolith sections made by two readers were contrasted. In addition, for each reader, ages estimated from fin spine sections were compared with ages estimated from otolith sections from the same fish.

Validation of annulus formation

The periodicity of annulus formation in Atlantic sturgeon fin spines and otoliths was studied by using measurements and chemical microanalysis of marginal increments, and observation of annuli in juvenile hatchery-reared sturgeon marked with oxytetracycline (OTC) at a known age. Efforts were concentrated on fin-spine age validation because annuli were difficult to consistently interpret in otolith sections.

Marginal increment analysis measures the opaque zone deposited after the last identifiable translucent zone at the margin of a structure used for age estimation (Kalish, 1995). Seasonal growth of that opaque zone is used to determine the timing of annulus formation (Cailliet et al., 1986; Beamish and McFarlane, 1987; Brennan and Cailliet, 1989). In our analysis, marginal increments were measured with image analysis software (Optimas, Inc., 1994) to the nearest 0.001 mm. Marginal increment ratio (MIR) was calculated as

$$MIR = MI \times 1/A,$$

where MI = the width of the outermost opaque zone (marginal increment); and
A = the mean width of the three annuli deposited previous to the marginal increment.

Mean MIR was computed for each month separately, with ages and sexes combined. Because very few fish were collected for several months, monthly samples were pooled for four three-month seasons. The winter season (December–February) was eliminated owing to very low sample size.

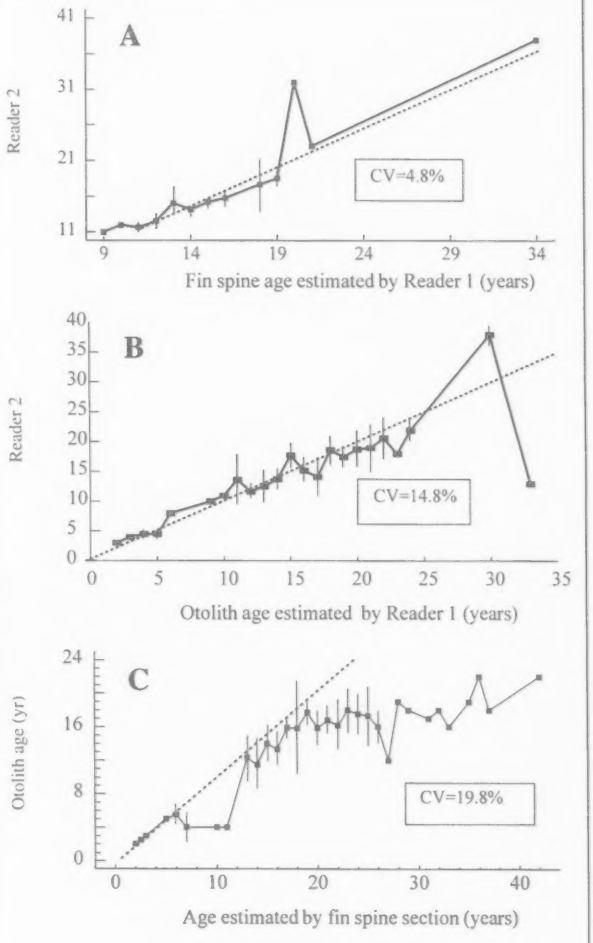

Figure 2

Bias in age estimates for Hudson River Atlantic sturgeon are evaluated by age-bias plots that compare estimates made by two readers interpreting the same (**A**) fin-spine sections and (**B**) otolith sections; and (**C**) made by one reader interpreting fin-spine and otolith sections taken from the same fish. Dashed lines are "equivalence lines" indicating the hypothetical case where both estimates are identical. Error bars represent the 95% confidence interval about the mean age (reader 2) for all fish assigned a given age by reader 1.

Microchemical analysis of calcified structures can verify the periodicity of annuli (Jones and Geen, 1977; Casselman, 1983; Radtke and Targett, 1984; Cailliet and Radtke, 1987). Calcium concentration is correlated with optically defined growth zones in the hard part (e.g. calcium is increased in the opaque zone and decreased in the translucent zone; Cailliet and Radtke, 1987; Lai et al., 1996). For instance, the most recently formed material in fin spines might contain high or low concentrations of calcium in fish collected during summer (rapid growth) or winter (slower growth), respectively (Cailliet and Radtke, 1987). In this instance, fin-spine microanalysis of calcium across annuli might show nadirs in calcium associated with translucent zones.

Calcium concentration in fin-spine sections was measured using a JEOL JXA-840A wavelength-dis-

persive (WDS) electron microprobe at the Center for Microanalysis, College Park, MD. Phosphorus was also measured because it makes up a large fraction of the fin spine's hydroxyapatite structure (Stevenson, 1997). Accelerating voltage was 25 kV and cup current was 20 nA. Each measurement represented rastering or scanning by the probe over a 5×5 µm area of the hardpart section. Molar weights of calcium and phosphorus were measured in peripheral regions of fin spines for 20 fish aged 3–18 years (fin-spine estimate) collected in March, June, September, and November. The mean of three peripheral points was calculated for each individual, and means were contrasted by month of collection with ANOVA.

Microprobe measurements were used to construct elemental chronologies of 15 fin spine sections taken from sturgeon aged 12–36 years (fin-spine estimate) and 164–236 cm TL. For each chronology, a series of point measurements was taken along an axis that traversed several annuli, with five points per annulus; points were assumed to sample seasons in linear proportion.

A solution of OTC (25 milligrams per kilogram of body weight) was injected into the dorsal musculature of five juvenile laboratory-reared fish of known age (55–65 cm TL) from Hudson River broodstock. Juveniles had been reared in circular fiberglass tanks in a recirculating system and fed a mixture of commercial pellet feeds. They were subjected to a twelve-hour photoperiod; water temperature ranged from 15° to 20°C. Three months after injection, the first fin spine section was removed from three fish with a jeweller's saw, and silver nitrate was applied to encourage clotting of the wound. Fifteen months after injection, a second section was removed from the opposite pectoral-fin spine of one fish only. Fin-spine segments were dried, sectioned, mounted on glass slides, and polished. Thin sections were viewed with epifluorescent microscopy to identify OTC marks. The position of OTC marks with respect to opaque and translucent zones was recorded and a micrograph was taken. In all cases, the reader was aware that the fish had received an injection of OTC but had no knowledge of the date of section collection.

Growth

Reported measurements of dressed carcasses were converted to total length based upon conversion metrics derived for Hudson River Atlantic sturgeon (Stevenson, 1997). Sturgeon included in the conversion metric sample (n=235) were collected during spawning season in the Hudson River; all but five possessed mature gonads. Ages derived from fin-spine and otolith sections were used to fit von Bertalanffy growth models by using a Marquardt iterative estimation procedure for the three model parameters. Length-at-age relationships were first examined to determine variance structure and progression of modal length with age. Growth parameters were estimated iteratively for males and females with a least-squares method. Because of high variance at the point of growth inflection, it was unlikely that a single growth model would fit all portions of the growth curve. Therefore, the juvenile portion (42–152 cm TL) was modeled separately with a power function based upon the best fit of residuals.

PC-SAS (SAS Institute, Inc., 1994) and Statgraphics Plus (STSC, Inc., 1992) were used for all statistical tests. Data that did not satisfy the assumption of heteroscedasticity (Bartlett's test, α=0.05) were transformed to satisfy this assumption. Transformed data that did not satisfy this assumption were analyzed with a Kruskal-Wallis nonparametric test to examine differences among groups.

Results

Comparison of hard parts

Otoliths were irregularly shaped and their annuli were difficult to interpret. In contrasting several sectioning planes, we observed that annuli on transverse sections yielded the most consistent interpretations. The first three to nine growth zones showed a clear alternation of opaque and translucent zones. Thereafter, translucent zones were irregularly spaced and often appeared to overlap (Fig. 1A). Low optical contrast between opaque and translucent zones reduced the readers' confidence in assigning annuli, especially in sections with more than twenty annuli.

In contrast, fin-spine sections exhibited concentric narrow translucent zones and wide opaque zones when viewed with transmitted light. Fin spines contained a vascularized core and deposits of organic material in lobe regions (Fig. 1B). Interspersed were fibrils that we interpreted as collagen or some other structural protein. Annuli became narrower toward the outer edge in larger (and presumably older) fish. Secondary fin spines (84% of fin-spine sections) and false annuli were observed but were simple to identify and disregard. Belts of two to five narrow annuli were apparent in most female fin-spine sections (96% of a subsample of 48). These belts were not apparent in the juvenile sturgeon examined.

Imprecision (CV) in age estimates was 4.8% between two readers of the same fin-spine section (Fig. 2A). Mean imprecision was 1.2 years (t=–1.97, P>0.05); estimates by the two readers were not significantly

different. Age estimates from otolith sections were less precise than from fin-spine sections (Fig. 2B). Absolute imprecision was 3.3 years and the CV on paired differences was 14.8%. No significant bias occurred in otolith interpretations between readers. In a comparison of ages estimated by a single reader from otolith sections and from corresponding fin-spine sections (i.e. from the same fish), the former were significantly lower than the latter (Fig. 2C; mean difference=5 yr, t=9.01, P<0.05). The bias was most apparent for presumed by older fish.

Validation of the fin-spine aging method

Marginal increment ratios for fish collected in late winter and spring (February–April) were significantly lower than those for summer and fall (Fig. 3; Kruskal-Wallis test; P=0.04). Mean MIR was 18% in spring, 33% in summer, and 36% in fall. The highest rate of marginal increment completion was observed for winter months (December–February), although sample size was very small for these months (n=3).

Readings of annuli from hatchery-reared sturgeon resulted in exact age estimates. The sturgeon were 4+ years old and annuli were clearly defined. There was a distinct difference in the shape of fin spines from hatchery-reared and wild juvenile sturgeon (Fig. 1C). Hatchery-reared fish exhibited irregularly shaped, compressed annuli which could indicate erosion or injury, but were nonetheless easily recognized. In OTC-injected fish, a distinct OTC mark followed by an opaque zone was apparent in all fin spines examined three months after injection. The fish did not show any symptoms of stress following fin-spine removal. The only sample examined at fifteen months after injection exhibited a clear OTC mark that was followed by opaque and translucent zones.

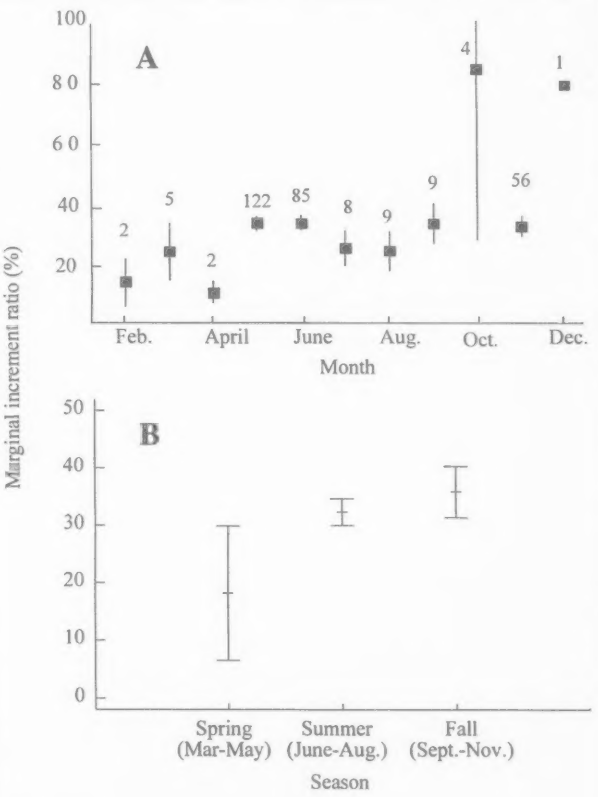

Figure 3

(**A**) Marginal increment ratios (MIR) ± one standard error for sectioned Hudson River Atlantic sturgeon pectoral fin spines evaluate current season's growth. Numbers represent sample size. (**B**) Marginal increment ± 95% confidence interval for data grouped by season. MIR = $MI \times 1/A$, where MI = the width of the outermost opaque zone (marginal increment) and A = the mean width of the three annuli deposited previous to the marginal increment.

Microchemical analysis

The concentrations of calcium and phosphorus in peripheral regions of fin spines showed significant seasonality (Kruskal-Wallis test, $P \leq 0.04$; Fig. 4). Fin spines collected in November were significantly lower in calcium and phosphorus than those collected in March, June, and September. Calcium-to-phosphorus ratios increased significantly from March through November (Kruskal-Wallis test, P=0.04). Plots of calcium and phosphorus in fin spines revealed cyclical trends in both elements, with peaks associated with translucent zones (Fig. 5).

Growth

The size-age relationship for prespawning fish, as judged by interpretations of annuli in fin spines, was best fitted by a power regression (r^2= 0.75; Fig. 6). Von Bertalanffy models for both males and females considerably underestimated length at age for subadults. Over all life history portions, the fit of the von Bertalanffy model was better for females (ages 2–42; r^2=0.56) than for males (ages 4–36; r^2=0.33) owing to the broader range of ages and lengths in our sample

of females (Fig. 7A). Males grew faster but reached a smaller asymptotic length at a younger age than did females. Females grew more slowly (K=0.07) toward a significantly larger maximum length (251 ±12.8 cm). The asymptotic growth phase for females and males was 12–42 and 11–28 years, respectively. Estimates of growth coefficients, K, were significantly different between sexes (t=73.2, df=431, P<0.05). Log transformation of the data did not correct for the error structure. Residuals from the models were designated as corresponding either to fish of the size at entry into the fisheries (152 cm TL in New York and after 1993 in New Jersey), or to larger or smaller fish. The resulting residual plots indicated that the faster-growing males were harvested just as they reached size at entry (positive residuals) and that the slower-growing females entered the fishery at much older ages (negative residuals; Fig. 7B). The modeled growth pattern for females appeared more biased than that for males owing to a sharp shift in residuals from positive to negative at about 15 years. At older ages, the von Bertalanffy model underestimated the size of females. These patterns in residuals were also apparent in males, albeit less pronounced.

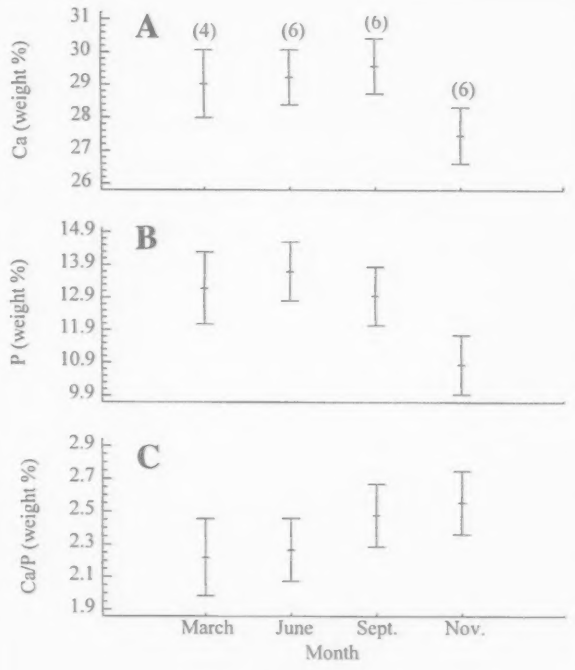

Figure 4

Microchemical analysis of marginal increments of fin spine sections taken from Hudson River Atlantic sturgeon show significant seasonality in (**A**) calcium; (**B**) phosphorus; and (**C**) calcium-to-phosphorus ratio. Means and 95% confidence intervals are depicted. Three points were analyzed per section. Numbers in parentheses indicate how many fin-spine sections were analyzed for fish collected in each of the four months (these apply to all three graphs).

Discussion

Fin spines or otoliths?

Owing to ease of collection and processing, as well as precision and accuracy in aging, we recommend the use of fin spines rather than otoliths for demographic analysis of Atlantic sturgeon. Preparation techniques used in this study (embedding, sectioning, and polishing) may have improved the visual resolution of annuli in pectoral-fin spines over that reported in earlier investigations.

Annuli in sturgeon otoliths and, as a result, growth rates, have been grossly misinterpreted in the past owing to examination of the external surface of hard parts only (Greeley, 1937). Especially in presumed older individuals, otoliths should not be used to verify age estimates based on fin spines. Annular clarity diminishes towards the distal end of the otolith (more recent growth), which may result in lower age estimates. In addition, individuals must be sacrificed to collect otoliths. Concern over declining sturgeon populations should restrict the size of otolith samples.

Precision and accuracy

Age estimates based on fin spines were unbiased and precise. Aging imprecision for Atlantic sturgeon was similar or better than imprecision reported for other long-lived (>20 yr) species, for which precision is often affected by narrow annuli, which result from reduced growth rates in older fish (Rien and Beamesderfer, 1994; Table 1). The annual patterns of marginal increment ratio (MIR), observations on fish of known age, and microchemistry have supported the hypothesis that a pair of opaque and translucent zones forms annually in Atlantic sturgeon fin spines. A small sample of OTC-marked juveniles also indicated annual annulus growth (Stevenson, 1997). Results for Atlantic sturgeon were similar to Huff's (1975) analysis of Gulf sturgeon (*Acipenser oxyrinchus desotoi*) fin-spine sections, in which a higher percentage of fin-spine sections showed that a completely formed

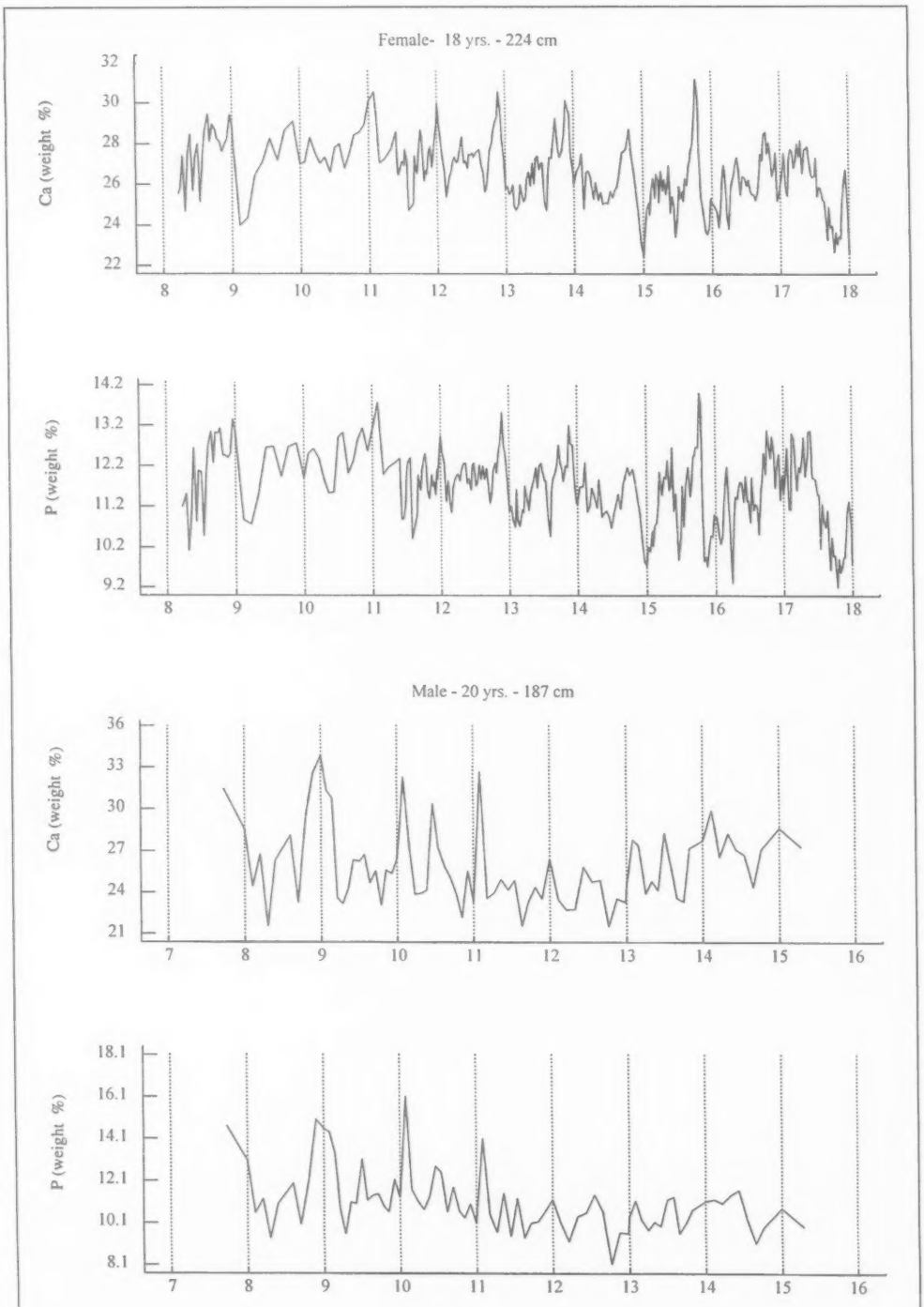

Figure 5

Series of microprobe analyses for calcium and phosphorus across annuli in fin-spine sections were used to construct elemental chronologies for Hudson River Atlantic sturgeon. In these two representative chronologies, dashed lines correspond with translucent zones.

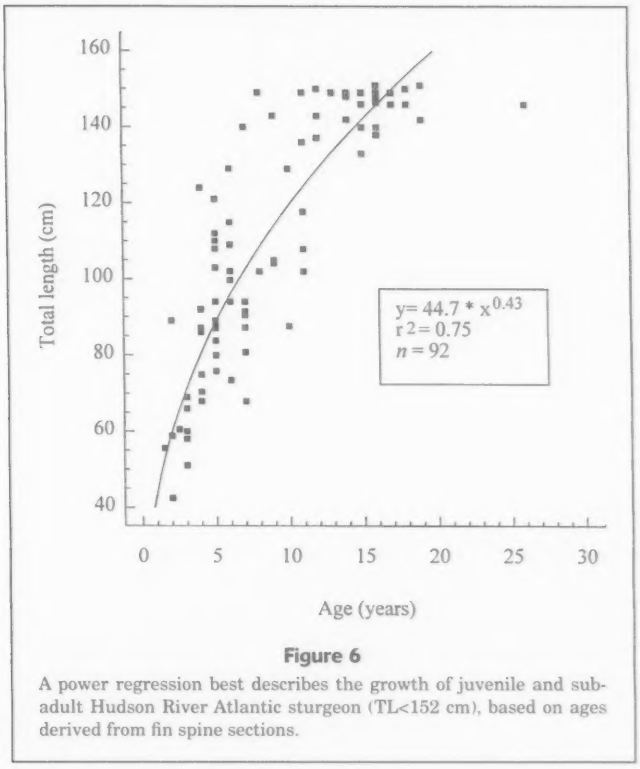

Figure 6

A power regression best describes the growth of juvenile and subadult Hudson River Atlantic sturgeon (TL<152 cm), based on ages derived from fin spine sections.

marginal annulus was completely formed in the fall rather than in the spring. Ideally, marginal increment analysis should be performed on separate age classes to ensure that annulus formation occurs for all ages (Casselman, 1983). However, owing to the relative scarcity of older individuals and the unavailability of fish in some seasons and because marginal increments are often difficult to discern in fin-spine sections taken from older individuals, age classes were pooled in our study. Accuracy of age estimation at older ages remains untenable. An attempt to validate longevity estimates of Hudson River Atlantic sturgeon using radiometric $^{210}Pb/^{226}Ra$ dating was unsuccessful (Burton et al., 1999). Recapture of hatchery-released Atlantic sturgeon (Secor[2]) has provided an opportunity to verify age determinations in older or mature individuals. Fin spines taken from juvenile sturgeon did not exhibit belts of annuli; such belts may provide information about spawning behavior in females.

Chemical microanalysis of hard parts is a promising tool for age verification (Jones and Geen, 1977; Casselman, 1983; Cailliet and Radtke, 1987). However, seasonal cycles were not consistently observed in elemental chronologies of Atlantic sturgeon fin spines. The technique assumes that the hard part is a closed system, and there is no resorption or remodeling. Burton et al. (1999) using radiometric tracers, found that Atlantic sturgeon fin spines appear to be open systems. In our study, we may have observed a corrupted seasonal signal.

Growth

Sexually dimorphic growth patterns in Atlantic sturgeon may be a result of differential reproductive schedules and migration patterns. The age-length relationship shows substantial variability in both males and females. Males mature earlier (12 yr) and spawn annually. Females mature later at a much larger size and are thought to spawn every 3–5 years (Smith, 1985). Lower growth rates and larger achieved sizes are typical for large, long-lived fish that broadcast numerous offspring (Adams, 1980; Moreau, 1987; Roff, 1988, Winemiller and Rose, 1992).

[2] Secor, D. H. 1998. Habitat utilization patterns of mid-Atlantic Bight juvenile Atlantic sturgeon. Final report on project 1445-CT-09-0189 to National Biological Survey, 30 p. Ref. no. [UMCES] CBL 98-019. [Available from David Secor, Chesapeake Biological Laboratory, 1 Williams St., Solomons, MD 20688-0038.]

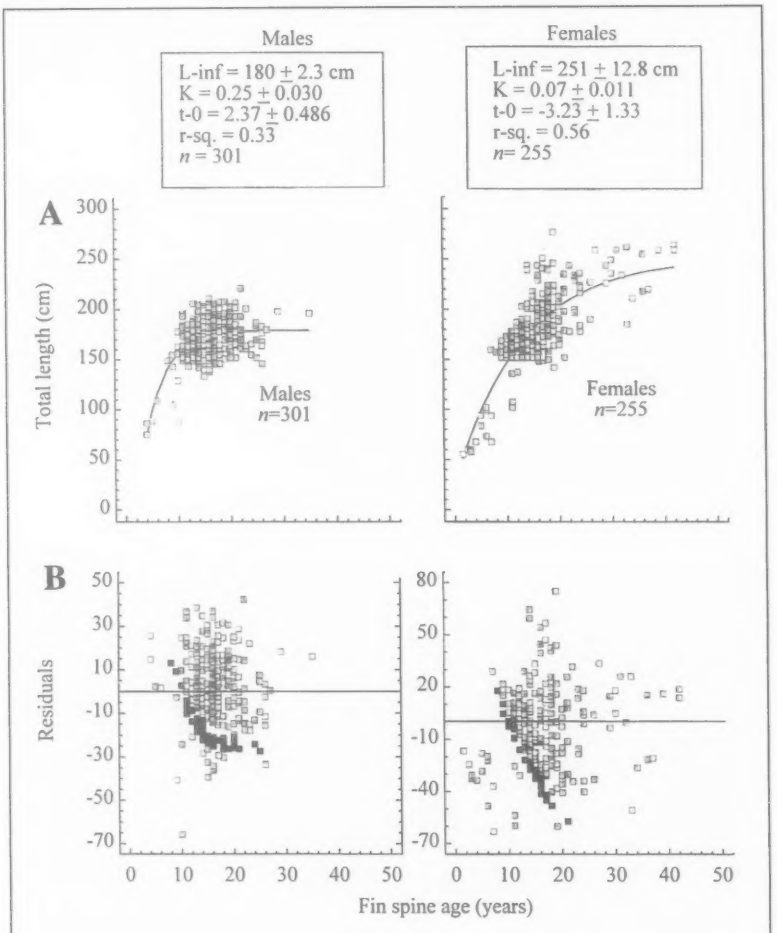

Figure 7

(**A**) Predictions of length at age from von Bertalanffy models for male and female Atlantic sturgeon (black curves) fitted observed length at age (grey squares) for females better. (**B**) Plots of residuals from the growth models reveal a difference between fish of size-at-entry (152 ±5 cm TL; black squares); and fish of TL above or below size-at-entry (gray squares). Note different *y*-axis scales for males and for females. Ages derived from fin-spine sections.

Growth is difficult to model accurately in long-lived species (Mulligan et al., 1987). Because little bias was detected in our readings (aging precision was unaffected by age), discrepancies in age estimates should not introduce systematic error in estimates of population parameters such as mortality rate, K, and L_∞. High variability in observed length at age indicates that length may be a poor predictor of age for Atlantic sturgeon. This high variability may result from amplification of early growth differences over a long life span. Variance in size at age may also have resulted from the nature of the Hudson River Atlantic sturgeon fishery. The imposed minimum size limit on Atlantic sturgeon commercial fisheries (>152 cm TL) may have biased the sample used for growth estimates and could have caused K and L_∞ to be over- and under-estimated, respectively (Fig. 8). The von Bertalanffy curve may have been driven upwards at younger ages by the potential maximum growth rate of the population. For instance, as soon as the fastest growing members of an age class exceeded the minimum size limit, they were harvested. At later ages, the curve is pulled downward as slower growing individuals enter the

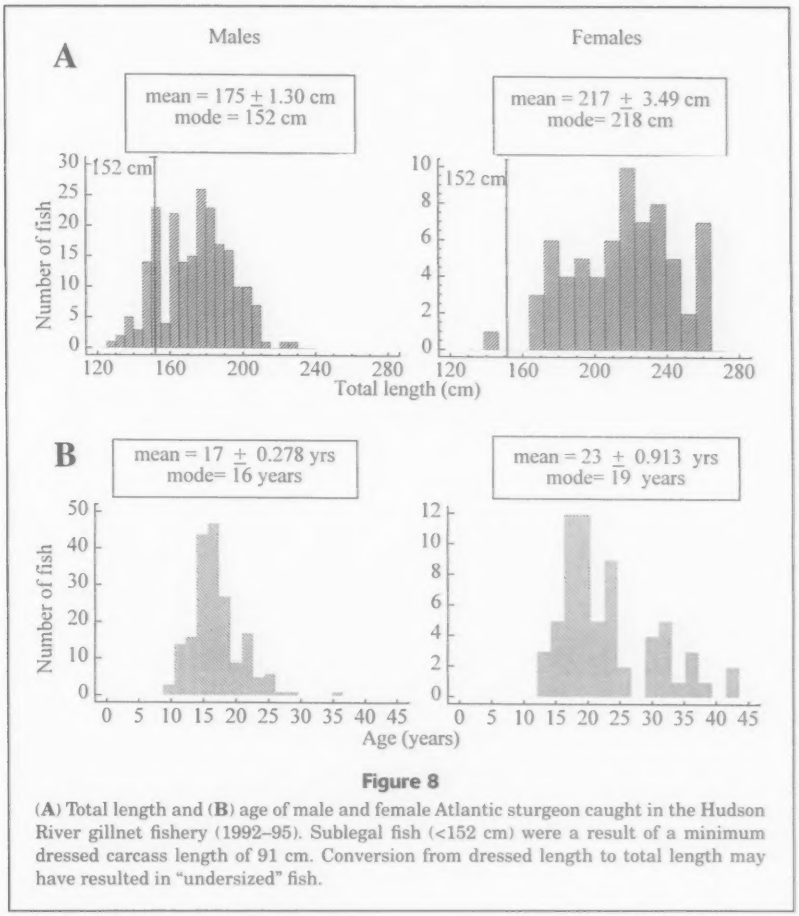

Figure 8

(**A**) Total length and (**B**) age of male and female Atlantic sturgeon caught in the Hudson River gillnet fishery (1992–95). Sublegal fish (<152 cm) were a result of a minimum dressed carcass length of 91 cm. Conversion from dressed length to total length may have resulted in "undersized" fish.

fishery (as evidenced by residual patterns), increasing K and decreasing L_∞.

Growth parameter estimates reported here for females are consistent with another study of the same population (Doroshov et al.[3]) but do not agree with an earlier study (with sexes combined) of the population (Dovel and Berggren, 1983). Recent reduction of the accumulated biomass by fishing, and resultant age and size truncation of the population, would cause lower apparent L_∞ and higher K values than in an unexploited population. Maximum length (L_∞) determined for females was substantially smaller than historical records of maximum size for this species (427 cm; Murawski and Pacheco, 1977), which may be a result of increased fishing pressure on the largest (female) component of the population during the past ten years. The historical longevity of females in the Hudson River also probably exceeded our estimate of 42 years; Magnin (1964) reported a 60-year-old female in the St. Lawrence estuary.

Because the largest male sampled (72 kg) was only 22 years of age, yet much smaller males were older than 30 years, male longevity in the unexploited population was also probably higher than in the population we sampled. Consideration of possible bias in von Bertalanffy growth parameters should direct managers to undertake sensitivity analyses in developing demographic-based stock assessments. Because L_∞ and K are inversely related (Kimura, 1980), age and size truncation would result in an over-estimated K (a lower L_∞ is approached more rapidly).

We found L_∞ values that were relatively low compared with estimates in previous studies of Hudson River Atlantic sturgeons (Greeley, 1937; Dovel and Berggren, 1983). These low values may indicate

[3] Doroshov, S., J. Van Eenennaam, G. Moberg, and G. Waton. 1994. Reproductive conditions of the Atlantic Sturgeon (*Acipenser oxyrinchus*) stock in the Hudson River. Report for year two to Hudson River Foundation. Animal Science Department, University of California, Davis, 65 p.

age truncation of the population by directed fishing effort. However, Dovel and Berggren (1983) reported difficulty in aging fish greater than 153 cm TL and may have misidentified annuli in both juveniles and adults. Greeley's (1937) study was based on enumerating annuli in otoliths. Likewise, K estimates from our study are higher than those estimated previously. Our growth estimates are consistent, however, with those of Doroshov et al.,[3] who aged the same population in the early 1990s.

Comparative studies of fish populations along a latitudinal gradient have shown an inverse relation between latitude and rates of growth and mortality (e.g. Leggett and Carscadden, 1978; Jennings and Beverton, 1991). Among Atlantic sturgeon populations, the most rapid growth was exhibited by fish sampled in southern latitudes. Maximum size increased with increasing latitude, which may indicate postponement of maturation (Magnin, 1964). Values of K for the Hudson River population did not differ significantly from those for any other population (α=0.05), whereas L_∞ values estimated for the Hudson River population were statistically different from those estimated for all other populations (α=0.05) along a latitudinal gradient (Table 2).

Table 2

Growth parameters have been calculated in various studies of Atlantic sturgeon populations (combined sexes). For Smith (1985), n=number of age classes. Smith's (1985) data were converted from fork length to total length (FL= 1.11×TL; Beamesderfer, 1993) and were presented as mean length by age class in the literature.

Location	Study	n	K	L_∞ (cm)
St. Lawrence River	Magnin, 1964	582	0.03	315
Kennebec, ME	Smith, 1985	7	0.06	236
Hudson River, NY	This study	634	0.08	225
Winyah Bay, SC	Smith, 1985	24	0.12	242
Suwannee River, FLA	Smith, 1985	17	0.14	184

Implications for stock restoration

Growth rates of acipenserid species are apparently affected by migratory behavior (Roff, 1988). Anadromous species of sturgeons exhibit higher growth coefficients than do semi-anadromous or freshwater resident species, although the semi-anadromous white sturgeon achieves a higher maximum length than do anadromous sturgeons (Table 3). Atlantic sturgeon undergo an ontogenetic habitat shift from estuarine nursery grounds to marine waters (Gilbert, 1989). We believe these species grow rapidly as juveniles (as shown by their high K coefficient) to outgrow predation in marine waters. Unfortunately, rapid growth may affect the timing of juvenile migration and increase their susceptibility to coastal fisheries at an early age.

Estimates of growth traits for Hudson River Atlantic sturgeon indicate that they are extremely vulnerable to overfishing. Slow growth following maturation, high longevity, and presumed low natural mortality indicate that year classes in this population reach their maximum biomass at relatively old ages (Alverson and Carney, 1975). Therefore, maximum fishing yield from sturgeon would be achieved at low

Table 3

Growth parameters (von Bertalanffy parameters unless otherwise indicated) estimated for North American sturgeons. Parameters were estimated for both sexes combined except where otherwise noted.

Species	Study	Location	n	L_∞ (cm TL)	K	t_0
Atlantic sturgeon (*A. oxyrinchus*)	This study[1]	Hudson River, NY	634	256 (female) 180 (male)	0.07 0.25	–3.2 2.4
White sturgeon (*A. transmontanus*)	DeVore et al., 1995	Unimpounded Columbia River, OR	783	307	0.03	–1.13
Green sturgeon (*A. medirostris*)	USFWS[1]	Klamath River, CA	173	238	0.05	2.0
Shortnose sturgeon (*A. brevirostris*)	Dadswell, 1979	St John's River, Canada	446	144[2]	0.04	2.0
Lake sturgeon (*A. fulvescens*)	Thuemler, 1985[3]	Menominee River, WI–MI	1464	156	0.07	–0.02

[1] Growth parameters estimated for males and females separately.
[2] United States Fish and Wildlife Service. 1982. Klamath River Fisheries Investigation Program annual report, p. 123–141.
[3] Converted from fork length (FL= 1.11 × TL; Beamesderfer, 1993).
[4] Growth parameters derived from plot of mean TL at age.

rates of fishing or harvest of large, mature individuals (Bullock et al., 1992; Stevenson, 1997). A moratorium on commercial sturgeon fishing in the New York Bight was instituted in 1996; however, Atlantic sturgeon continue to be caught incidentally in many coastal fisheries. Continued research and monitoring of recruitment and bycatch mortality will be necessary to ensure the restoration of this population.

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Settlement and nursery habitats for demersal fishes on the continental shelf of the New York Bight*

Brian P. Steves
Marine Sciences Research Center
State University of New York at Stony Brook
Stony Brook, New York 11794-5000
Present address: Marine Invasions Laboratory
Smithsonian Environmental Research Center
648 Contees Wharf Road
Edgewater, Maryland 21037
E-mail address: Steves@serc.si.edu

Robert K. Cowen
Marine Sciences Research Center
State University of New York at Stony Brook
Stony Brook, New York 11794-5000

Mark H. Malchoff
New York Sea Grant Program
3059 Sound Avenue
Riverhead, New York 11901

Abstract.—Although juvenile fish are studied extensively in estuarine and nearshore environments, surprisingly little is known about the basic habitat requirements of juveniles for offshore settlement and nursery areas. Between June 1996 and July 1997, settlement and nursery habitats of age-0 (early juvenile) demersal fish on the continental shelf of the New York Bight were investigated by using a two-meter beam trawl. Replicate tows at 21 stations along three cross-shelf transects (20–95 m depth), were sampled on a near monthly basis to determine general ecology (21,309 fish collected in 659 tows). Of the 47 species collected, 33 included age-0 juveniles, and 25 included near-settlement size individuals. The two dominant species, *Pleuronectes ferrugineus* and *Merluccius bilinearis*, constituted 88.9% of the total catch of age-0 fish. Of all age-0 fish, 94% were collected during summer and fall. Comparisons of weighted means and the use of canonical correspondence analysis determined that settlement and nursery habitats across the shelf are primarily delineated by depth, temperature, and time of year. Three zones across the shelf (inner, middle, and outer) each had distinct juvenile fish assemblages. Knowledge gained about the distribution and quality of juvenile habitat for commercially important offshore species should facilitate their improved management.

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With the decrease in fish abundance in the latter half of the twentieth century, particularly in the Northwest Atlantic (McHugh, 1972; NEFSC, 1992), fisheries managers have been concerned with both overfishing and habitat degradation. Much effort has been put into understanding the abundance, distribution (Colton, 1972; Colvocoresses and Musick, 1984), and environmental preferences (Scott, 1982; Auster et al., 1991; Felley and Vecchione, 1995) of adult groundfishes in the northwestern Atlantic. Although information on adult groundfishes is useful, events during the early life history of fish may be more important in determining recruitment variability (Sissenwine, 1984; Houde, 1987; Peterman et al., 1988; Bradford, 1992; Miller, 1994). Several ichthyoplankton surveys have helped to increase our understanding of egg and larval distributions of groundfishes in the Mid-Atlantic Bight (MAB; Morse et al., 1987; Cowen et al. 1993). However, less is known about the juvenile stage, which represents a dramatic change in lifestyle for groundfishes: they leave the three-dimensional environment of the plankton and settle onto the two-dimensional world of the sea floor (Chambers and Leggett, 1992).

Within this two-dimensional environment, the growth, survival, and recruitment of groundfishes are affected by various factors associated with the quality (value for growth) and quantity (area) of their nursery habitat (Gibson, 1994). Research involving the nurseries of groundfishes has been limited to estuaries and nearby coastal habitats where they can readily be studied (Riley et al., 1981; Able et al., 1989; Bolle et al., 1994; Henderson and Seaby, 1994; Nash et al., 1994;

* Contribution 1179 of the Marine Sciences Research Center, State University of New York at Stony Brook, Stony Brook, NY 11794-5000.

but see Toole et al., 1997). However, Able et al. (1989) have suggested that there is a lack of studies that examine the continental shelves of the MAB as potential nursery grounds. This is remarkable because the early life history stages of many species of groundfish are believed to inhabit almost the entire shelf as well as the slope (Fahay, 1983; Miller et al., 1991).

A wide assortment of biotic and abiotic variables may have a role in determining the distribution of continental shelf nursery grounds. Variations of abiotic parameters, such as temperature, salinity, and oxygen, can affect the metabolism of marine flatfishes during the juvenile stage (Malloy and Targett, 1991; Pihl et al., 1991; Gibson, 1994; Neill et al., 1994). Changes in temperature, for example, can have a marked effect on feeding rate and growth; thus lower temperatures may slow growth and consequently increase susceptibility to mortality due to size-selective predation (Van der Veer et al., 1994). Salinity seems to have a small influence on the growth rate of fish, but it is often effective in controlling their distribution (Rodgers, 1992). By adjusting their position in relation to local abiotic factors, fish may modify their growth rate and survival with respect to average environmental parameters (Gibson, 1994).

Biotic factors, particularly food resources and shelter related to biological activity, may also be a potent determinant of nursery habitat. Auster et al. (1991) found that several biotic microhabitat types, including amphipod tube mats, shells, and biogenic depressions, significantly affected the abundance of fishes and other megafauna. Other biotic factors that influence the distribution of juvenile fishes include the presence of potential predators (Bailey, 1994) and the availability of potential prey species. Biotic factors may be ephemeral; however their presence or absence at a given time and space may control the distribution of associated fish species. The spatial distribution of amphipod tubes in particular have been shown to have a strong influence on the abundance of age-0 silver hake (*Merluccius bilinearis*) during fall in the MAB (Auster et al., 1997).

Recent recruitment research has emphasized factors that affect survival of the early life history stages of fishes. Identification of the habitats preferred by juvenile fishes in offshore waters will allow for a more complete understanding of recruitment variation. Although shelf habitat is magnitudes larger in area than the nearshore habitat traditionally studied, there is currently little knowledge of its role as a nursery. For many flatfish species, the size of nursery habitat and year-class strength have been correlated (Rijnsdorp et al., 1992; Gibson, 1994). Our objectives were to provide a first-order analysis of the species that use the shelf as settlement and nursery habitat during the course of a year and to address the relation of these distributions to environmental correlates.

Description of the study area

The New York Bight (NYB), a central portion of the MAB, encompasses an area of 39,000 km^2 on the East Coast of the United States. Its boundary extends out to the 200-m isobath between Montauk Point, New York, and Cape May at the southern end of New Jersey. The bathymetry of the NYB varies on several geographical scales. Although the continental shelf in this area is, to the most extent, gently sloping, large-scale features do exist. The Hudson River Canyon, which almost bisects the NYB, is the most obvious of these. Imposed on this bathymetry are the convoluted isobaths of the ridge and the swale topography that dominates the NYB (Freeland and Swift, 1978). Surficial sediments range from fine sand along the southern shore of Long Island to pebbly gravel off the coast of New Jersey (Schlee, 1964). On a somewhat smaller scale, the sediments vary in color from yellow ochre to greenish gray and in composition from biogenic calcium carbonate to quartz and feldspar (Freeland and Swift, 1978).

The hydrography of the NYB changes seasonally. During the winter months, the entire water column is well mixed, but the coldest waters are found nearshore (Bowman and Wunderlich, 1977). Stratification begins in the spring and peaks in the summer. During stratification, a body of cold dense water, known as the cold pool, remains trapped on the bottom under the pycnocline on the midshelf. This cold pool forms a distinct band of midshelf temperature minima (about 4–5°C in midsummer) from Georges Bank to Cape Hatteras, persisting from late spring to early autumn (Houghton et al., 1982). Outside the cold pool, bottom temperatures in the NYB range inshore from less than 1°C in the winter to above 21°C in the summer. Deeper bottom water, near the 100-m isobath, is less variable, ranging from 7° to 12°C (Ketchum and Corwin, 1964). Salinity in the NYB varies less, with only a mild seasonal cycle. The lowest salinities (<31 psu) are found near the apex of the NYB, southeast of the Hudson River. However, salinities as high as 35 psu are found at the 200-m isobath. As with temperature, the largest seasonal fluctuations in salinity are found nearshore (Bowman and Wunderlich, 1977). Overall, nearshore habitats are less stable than those offshore in terms of both salinity and temperature.

Materials and methods

Sample collection

Ten sampling cruises were conducted between June 1996 and July 1997 on board an 85-foot commercial fishing vessel, the *Illusion*. Summer and fall cruises were conducted monthly, and winter and spring cruises were conducted every other month (Table 1). The 21 stations sampled during each cruise were arranged in three transects (west, central, and east), each with seven stations ranging in depth from about 20 meters to 90 meters (Fig. 1). Stations on each transect were located according to bathymetry so that six stations were distributed evenly across the range of depths (one approximately every 15 m of depth). The seventh station was placed to fill any large distance between stations that was due to variability in the slope. The distance from shore of stations at a particular depth varied between transects.

Not all stations were sampled during every cruise (Table 1). Owing to weather, only five stations were sampled during cruise 6 (December 1996) and only two of the three transects were sampled during the February 1997 and April 1997 cruises. Because less than 25% of the stations were sampled during cruise 6, we did not include these data in some of the analyses. However, cruises 7 and 8 were included because of their broader range of coverage (between the two cruises all three transects were covered at least once).

Temperature and salinity data were collected after trawling at each station by using an internally recording conductivity-temperature-depth (CTD) probe (Applied Microsystems Inc. model AMS-STD 12). Care was taken to collect data from as close to the bottom as possible without risking damage to the CTD. Because a backup CTD was unavailable and the CTD used was not always reliable, gaps in the physical data exist. Expendable bathythermograph (XBT) data collected nearby in early August by the MV *Oleander* as part of the NOAA Ship of Opportunity Program (SOOP) were substituted for missing temperature data for the central transect in that month.

Juvenile groundfishes were collected with a modified 2-m beam trawl with 4-mm stretch mesh net (and 5-cm stretch mesh outer net for chaffing), towed at about 2–2.5 knots. The addition of a meter wheel allowed us to measure the area swept as the distance trawled, multiplied by the width of the trawl (as in Carney and Carey, 1980). At each station, three 5-min tows were made; a fouled trawl was discounted and another trawl was repeated. The mean area swept during a 5-min tow was 698 m^2 ±35.1 m^2 (95% CI).

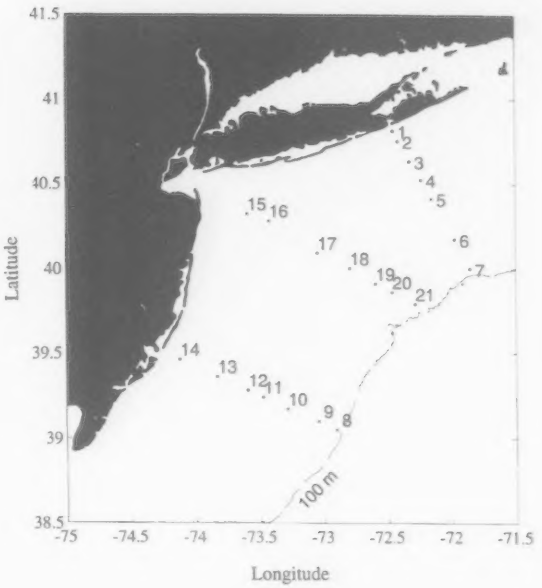

Figure 1

Twenty-one stations (numbers) on the continental shelf of the New York Bight were sampled with a two-meter beam trawl.

Table 1

Dates and sampling stations in the New York Bight for each of the ten cruises in this study. For each of the three transects, numbers indicate the stations sampled on a given cruise (sample locations are depicted in Figure 1).

Cruise number	Dates	West transect	Center transect	East transect
1	14–17 Jun 1996	8–13	16–21	1–7
2	9–11 Jul 1996	8–14	15–21	1–7
3	9–11 Aug 1996	8–14[1]	15–21[2]	1–7[1]
4	20–22 Sep 1996	8–14	15–21	1–7
5	25–27 Oct 1996	8–14	15–21	1–7
6	10 Dec 1996	—	—	1–5
7	26–27 Feb 1997	8–14	—	1–7
8	16–17 Apr 1997	8–14	15–21	—
9	11–13 Jun 1997	8–14	15–21	1–7
10	15–17 Jul 1997	8–14	15–21	1–7

[1] During the August cruise, no temperature or salinity data were collected.

[2] The center transect was supplemented by XBT data.

A 2-m beam trawl is the recommended standard trawl for juvenile groundfish research because its fixed width allows for ready quantification of the area trawled (Kuipers et al., 1992). Our trawl had skids that were heavier than those on most stan-

dard 2-m beam trawls in order to reduce the amount of time the trawl took to reach the bottom and to ensure that it was heavy enough to remain there. To accommodate the increased weight (about 38 kg) and prevent the trawl from digging into the sediment, the skids were also longer (0.85 m) and wider (0.1 m). Typically, 2-m beam trawls are not used in depths greater than seven meters (Rodgers, 1992; Gibson, 1994; but see Pearcy, 1978); however, these modifications enabled us to trawl easily in waters as deep as 100 m.

Fish samples were sorted on deck and all recently settled juvenile fishes (age-0) were preserved in 95% denatured ethanol. These fish were measured in the laboratory to the nearest tenth of a millimeter standard length (SL) with digital calipers. Older fish were measured on deck to the nearest millimeter and returned to the water. With the exception of *Sebastes* and *Urophycis* spp., all age-0 fishes were identified to species. Fish stages were identified according to morphological changes such as squamation and, for flatfish, eye migration, as well as analysis of length frequencies of cohorts. The relative abundance and composition of the remaining sample (shell hash, sand dollars, and shrimp) were estimated from subsamples.

Length-frequency distributions of each species collected were calculated for each cruise. To account for differences in sampling effort among cruises, abundance data were standardized to the average cruise, which had 63 tows (21 stations × 3 tows each) lasting five minutes each. Length frequencies were then used to estimate the size range of the age-0 fishes collected during each cruise, as well as to infer some growth rates of the age-0 cohort between cruises.

Distribution analysis

Abundance data were standardized to the average number of fish per 1000 m^2. Because we were specifically interested in early juveniles, and adults were not efficiently collected, we limited our analysis to age-0 fishes. All species of age-0 fishes collected were included in the analyses. The distribution of age-0 fishes was evaluated with respect to a suite of environmental data (bottom temperature, bottom salinity, and depth) by comparing weighted means for each species (Scott, 1982). For a given species, an environmental variable such as temperature was weighted by the abundance of that species at each sampling station. The sums of each weighted variable were then divided by the total abundance of that species collected to yield a mean value for that species on that environmental parameter.

Cross-shelf migrations with ontogeny are a common feature of demersal fishes, particularly flatfish (Riley et al., 1981; Toole et al., 1997). To determine changes in cross-shelf distribution with size, weighted mean depths by size class were calculated for the more abundant species collected. In particular, distinct differences between the location of near-settlement size fishes and larger age-0 fishes might indicate migration between settlement and nursery habitats.

Environmental correlates

Distribution and abundance of fishes in relation to underlying multivariate environmental gradients were analyzed by canonical correspondence analysis (CCA) by using the software program CANOCO (ter Braak, 1992). This analysis has been widely applied in the field of community ecology (ter Braak, 1995; ter Braak and Verdonshot, 1995; Rakocinski et al., 1996); it entails reciprocal averaging of species and environmental data based on the assumption of a unimodal response of species abundance to the environment (Palmer, 1993; ter Braak, 1995). In a comparison of ordination techniques, Palmer (1993) found that CCA was robust, being less susceptible to spurious results such as the "arch effect" often common to principal components analysis (PCA). Furthermore, Palmer's (1993) simulations of CCA illustrated that noisy or skewed species data could be compensated for, and a variety of data types and sampling design were possible.

In total, 25 environmental variables (Table 2) were sampled during the cruises and included in the CCA analysis. Bottom temperature and salinity (from CTD casts), station depth (from an onboard depth sounder), latitude and longitude (global positioning system [GPS]), and distance from shore (nautical charts) were employed as continuous measures during the analysis. The relative abundances of nonfish constituents collected by the trawl were also considered to be environmental variables. Information on surficial sediment character at each station, another environmental variable we employed, was obtained from published data from the Marine EcoSystems Atlas (MESA) program's New York Bight Project (Freeland and Swift, 1978). Although these data are independent of this study, the MESA sampling area was the same and the spatial resolution of its sediment sampling was fine enough to obtain general information suitable for our analysis. The data on nonfish trawl constituents and surficial sediments were entered into the analysis as scaled values (i.e. as a number describing relative abundance among stations and tows). Because collections of both species and environmental data were made year-round, seasonal variables were also included and coded in the analysis as a set of nominal variables: spring (March–May), summer (June–August), fall (September–November),

Table 2
List of environmental variables used in the canonical correspondence analysis (CCA).

Environmental variable	Code	Type	Units
Spring	Spr	seasonal	true-false
Summer	Sum	seasonal	true-false
Fall	Fall	seasonal	true-false
Winter	Win	seasonal	true-false
Latitude	Lat	physical	° latitude
Longitude	Long	physical	° longitude
Bottom temperature	T	physical	° Celsius
Bottom salinity	Sal	physical	psu
Distance from shore	D	physical	km
Depth	Z	physical	m
Median grain size	MePhi	physical	Φ-scale
% organics in sediment	%Org	physical	percent
% calcium carbonate in sediment	%CaCO3	physical	percent
Northern sea stars, *Asterias forbesi*	noss	biological	relative volume of sample
Sand dollars, *Echinarchnius parma*	snd	biological	relative volume of sample
Margined sea stars, *Pontaster tenuispinus*	marg	biological	relative volume of sample
Small shell fragments	hash	biological	relative volume of sample
Large mollusk shells and shell pieces	shell	biological	relative volume of sample
Sea scallops, *Placopecten magellanicus*	scall	biological	relative volume of sample
Sand shrimp, *Crangon septemspinosa*	crang	biological	relative volume of sample
Boreal shrimp, *Pandalus montagui*	borsh	biological	relative volume of sample
Rock crabs, *Cancer* spp.	crab	biological	relative volume of sample
Amphipods	amph	biological	relative volume of sample
Fig sponges, *Suberites ficus*	spon	biological	relative volume of sample
Large biogenic tubes	tube	biological	relative volume of sample

and winter (December–February). Each environmental variable was standardized to have a mean of zero and a standard unit of variance so that all variables had equal weight despite differences in the scales of their usual units.

Although seasonal patterns are likely to be important in juvenile fishes, these patterns are likely to be influenced by the spawning patterns of adult fishes rather than by habitat selection of settling fishes. These seasonal effects may dilute the variation explained as due to other factors. Some environmental factors such as temperature, salinity, and the presence of ephemeral biota that vary with season are possibly of more interest than simply accounting for settlement timing. To this end, a partial CCA was conducted using season as a covariable. Partial CCA allows environmental variables to be treated as covariables, thus removing their influence from the rest of the data set (ter Braak and Verdonschot, 1995).

A forward selection of environmental variables was used to select a minimum set of environmental variables that best explained the distribution and abundance of age-0 fishes (ter Braak, 1992). Forward selection begins by the selection of the variable that explains the most variation in the data set. This variable is then treated as a covariable, the data set is then reevaluated, and the next most important variable is selected. This procedure continues until the desired subset of variables has been selected. To test the advisability of adding variables, Monte Carlo permutation simulations are conducted. Variables are added as long as their addition continues to contribute significantly to the explained variance ($P<0.05$). Forward selection was performed on the data set, with season treated as a covariable. The combination of partial CCA and forward selection is not considered problematic with regard to the assumptions of the analysis (ter Braak, 1992).

Results

Faunal composition and distribution

A total of 21,309 fish representing 47 species and 32 families were collected over the course of the ten cruises and the 659 tows. Of these 47 species, 33 were collected as age-0 juveniles, including 25 spe-

Table 3

Minimum, mean, and maximum standard length (mm) of the 32 species of age-0 demersal fish collected. Maximum larval size (Fahay, 1983) and sample size (n) are also indicated. Maximum sizes estimated from size-frequency distributions are included (see Figs. 4–6). Species are ordered by decreasing total abundance. An asterisk next to minimum standard length indicates that the species was collected at near-settlement size.

Species	Species code	Max larval size (mm)	Min. SL (mm)	Avg. SL (mm)	Max. SL (mm)	n
Pleuronectes ferrugineus	PLEFER	11–16	5.7*	17.4	34.9	10,452
Merluccius bilinearis	MERBIL	17–20	12.6*	28.2	145.0	3,195
Liparis inquilinus	LIPINQ	12–13	9.6*	20.4	49.0	595
Raja erinacea	RAJERI	80–90	87.2*	117.6	146.4	281
Urophycis spp.	UROSPP	20–25	8.8*	32.5	49.0	224
Macrozoarces americanus	MACAME	30	32.0*	89.1	119.0	213
Citharichthys arctifrons	CITARC	13–15	10.8*	18.8	29.7	120
Lepophidium profundorum	LEPPRO	20	85.5	85.5	115.0	117
Ophichthus cruentifer	OPHCRU	83.5	70.0*	111.0	146.0	80
Gadus morhua	GADMOR	20–30	20.0*	36.8	49.0	62
Peprilus triacanthus	PEPTRI	12–18	6.8*	15.2	45.0	61
Glyptocephalus cynoglossus	GLYCYN	22–35	13.5*	30.0	47.5	40
Helicolenus dactylopterus	HELDAC	20	21.0*	28.9	35.0	38
Enchelyopus cimbrius	ENCCIM	20	9.1*	26.7	47.0	35
Etropus microstomus	ETRMIC	10–12	14.7*	23.6	29.0	26
Prionotus carolinus	PRICAR	20	11.1*	30.1	49.0	26
Myoxocephalus octodecemspinosus	MYOOCT	15	15.9*	22.2	44.0	22
Scophthalmus aquosus	SCOAQU	9	9.1*	59.8	143.0	19
Paralichthys oblongus	PAROBL	10–12	9.4*	15.8	37.0	15
Hippoglossoides platessoides	HIPPLA	18–34	20.1*	23.7	26.1	13
Ophidion marginatum	OPHMAR	35–50	52.1*	64.4	75.3	10
Stenotomus chrysops	STECHR	17	52	64.4	75	10
Sebastes spp.	SEBSPP	24	25.5*	28.1	33.1	8
Lophius americanus	LOPAME	50	56.0*	74.2	96.5	5
Melanogrammus aeglefinus	MELAEG	20	39.0	44.7	48.0	4
Centropristis striata	CENSTR	8.3	25.0	29.8	35.5	3
Hemitripterus americanus	HEMAME	19	27.0	28.0	29.0	2
Astroscopus guttatus	ASTGUT	20	9.8*	9.8	9.8	1
Monolene sessilicauda	MONSES	33	41.0	41.0	41.0	1
Pholis gunnellus	PHOGUN	30–35	39.0*	39.0	39.0	1
Tautogolabrus adspersus	TAUADS	8	8.7*	8.7	8.7	1
Citharichthys cornutus	CITCOR	13–15	53.2	53.2	53.2	1
Pleuronectes americanus	PLEAME	10–13	81.2	81.2	81.2	1

cies collected at near-settlement sizes on the shelf (Table 3). Two species represented 88.9% of the age-0 fishes collected: yellowtail flounder (*Pleuronectes ferrugineus*, 67.8%) and silver hake (*Merluccius bilinearis*, 21.1%). Overall, densities of these dominant species on the shelf during peak settlement averaged 55.7 and 26.23 per 1000 m^2, respectively (Table 4), although maximum densities of 771.4 and 660.9 per 1000 m^2, respectively, occurred during peak settlement in individual tows. In addition to age-0 juveniles, both larval (n=267, <2% total), and adult (n=2765, 13.0% total) fishes were captured with the trawl. Of the 25 species collected at or near-settlement size, 11 included individuals smaller than predicted settlement size, based on published maximum larval size (Fahay, 1983).

Most (94%) age-0 fishes were collected in the summer and fall (Table 4). *P. ferrugineus* and *M. bilinearis*, the two dominant species, settled almost exclusively during summer and fall, respectively (Fig. 2). However, if species richness is considered rather than species abundance, summer and fall are still the primary

Table 4

Mean seasonal density (fish/1000 m^2) of 21 abundant age-0 fishes over the entire continental shelf of the New York Bight. Winter and spring cruises are combined. Species are grouped by their season of highest density and only species with a density greater than 0.1 in at least one season are included.

	Summer 1996	Fall 1996	Winter 1996–Spring 1997	Summer 1997
Summer				
Pleuronectes ferrugineus	55.68	0.53	—	1.29
Citharichthys arctifrons	3.84	2.25	0.66	0.09
Macrozoarces americanus	0.94	0.36	0.16	0.20
Gadus morhua	0.40	0.03	0.14	—
Helicolenus dactylopterus	0.09	0.03	—	0.26
Peprilus triacanthus	0.24	0.04	—	0.17
Glyptocephalus cynoglossus	0.21	0.03	—	0.05
Enchelyopus cimbrius	0.21	0.03	—	0.05
Myoxocephalus octodecemspinosus	0.11	0.07	0.11	0.09
Fall				
Merluccius bilinearis	0.07	26.23	1.04	0.22
Liparis inquilinus	1.19	1.89	0.09	1.75
Urophycis spp.	0.26	1.88	1.05	0.07
Lepophidium profunforum	0.09	0.77	—	—
Ophichthus cruentifer	0.02	0.61	—	0.11
Etropus microstomus	0.11	0.40	0.79	0.05
Paralichthys oblongus	0.06	0.13	—	0.05
Winter–Spring				
Raja erinacea	0.60	0.56	0.90	0.54
Prionotus carolinus	0.04	0.22	0.46	—
Centropristis striata	—	0.15	0.20	—
Ophidion marginatum	—	—	0.11	—
Scophthalmus aquosus	0.02	0.07	0.11	0.05

time of settlement on the shelf (Fig. 2). Although many species settled between August and October, *Macrozoarces americanus* and *Gadus morhua* settled, and *Raja erinacea* hatched, on the shelf starting in midwinter.

Juvenile fish were colleced at all 21 stations sampled. Mean depths of all species of age-0 fish, weighted by abundance, are shown in Figure 3. Although the depth distributions cover the entire depth range of the surveyed shelf, for convenience of discussion we separate species into three depth groups (inner, middle, and outer shelf; Table 5). Inner shelf species included two flounders (*Etropus microstomus* and *Paralichthys oblongus*), searobin (*Prionotus carolinus*), and fourbeard rockling (*Enchelyopus cimbrius*). Little skate (*Raja erinacea*) was collected on the inner shelf at sizes near to hatching size. Midshelf settlers were dominated by *Pleuronectes ferrugineus* but also included large numbers of inquiline snailfish (*Liparis inquilinus*) and phycid hakes (*Urophycis* spp.). The dominant fish that settled on the outer shelf were silver hake (*Merluccius bilinearis*), Gulf Stream flounder (*Citharichthys arctifrons*), margined snake eel (*Ophichthus cruentifer*), fawn cusk eel (*Lepophidium profundorum*), and black-bellied rose fish (*Helicolenus dactylopterus*).

Generally, distributions along depth (Fig. 3A) and salinity (Fig. 3B) gradients showed similar but inverse trends; this finding was expected because of high correlation between bottom depth and bottom salinity. Weighted average distributions with respect to bottom temperature (Fig. 3C) did not show similar trends; seasonal variation in temperature and the presence of minimum temperatures midshelf during the summer precludes temperature and depth trends from being similar. Midshelf species such as *P. ferrugineus* and *Liparis inquilinus*, which settle in summer, had the coldest mean temperatures of collection. Outer-shelf species found in slope water had slightly warmer preferences, and those of inner-shelf species were even warmer.

Individual year classes were distinct, based on length frequencies for the more abundant species (Fig. 4–6). For several species (e.g. *P. ferrugineus* and

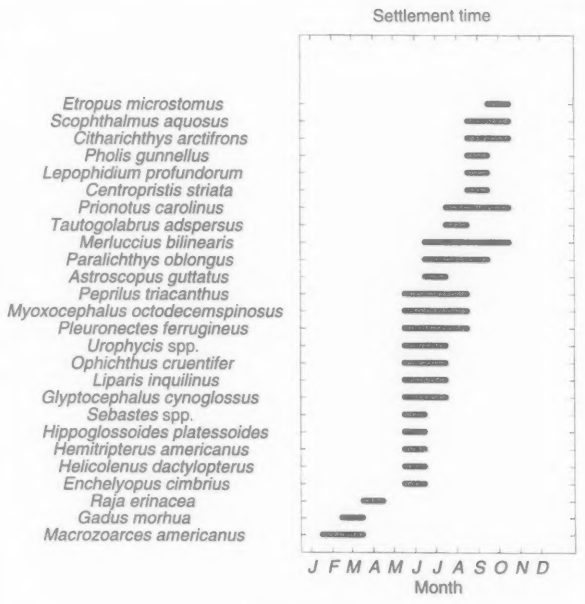

Figure 2

Timing of settlement for age-0 fish collected. For each species, a black horizontal bar marks the months in which cruises collected fish at or near settlement size. In some months there was no cruise (Table 1), and any settlement that might have occurred was not witnessed.

M. bilinearis), large decreases in abundance were observed following initial settlement, whereas abundance decreased at a more modest rate for other species such as *C. arctifrons*, *Macrozoarces americanus*, and *L. inquilinus*.

For species such as *P. ferrugineus* and *L. inquilinus* that settled during the summer (Fig. 4), the cohort could be followed for the entire first year after settlement (June 1996–July 1997). For other species that did not settle in the summer (Figs. 5 and 6), the cohort from the previous year was observed until the new cohort settled in the fall, winter, or spring. Inferred growth rates from the length-frequency distributions varied from two millimeters per month for species such as *P. ferrugineus* and *L. inquilinus* (Fig. 4), to about 15 mm per month for *Merluccius bilinearis* (Fig. 5) and *Macrozoarces americanus* (Fig. 6).

Cross-shelf movement between and within settlement and nursery areas was evident for some species but not for others. *Pleuronectes ferrugineus*, *Etropus microstomus*, and *Lepophidorum profundorum* are examples of species with consistent mean depths of distribution during their first year after settlement (Fig. 7, A–C). However, *M. americanus* exhibited a gradual migration towards deeper waters, whereas *C. arctifrons* and *Merluccius bilinearis* moved rapidly after settlement to waters about 30 meters deeper for the remainder of the first year (Fig. 7, D–F).

Habitat characteristics

Bottom temperatures in the NYB were dynamic (Fig. 8), and seasonal shifts in temperature were more varied at nearshore stations (5–20°C at the 25-m isobath) than at offshore stations (7–11°C at 90 m). Midshelf bottom temperatures showed a moderate seasonal range (4.3–14°C at 50 m). During the summer, bottom temperatures were stable, and there was only a slight increase of about 1°C per month. The highest rate of increase in bottom temperatures was recorded at the inshore stations: 8°C between August and September, associated with an early fall turnover in 1996. This was followed by the highest rate of cooling (–3°C) at the same nearshore locations between the September and October cruises. During fall turnover, the bottom temperatures increased by about 4°C per month over the midshelf, for a total of 8°C in two months. Following this

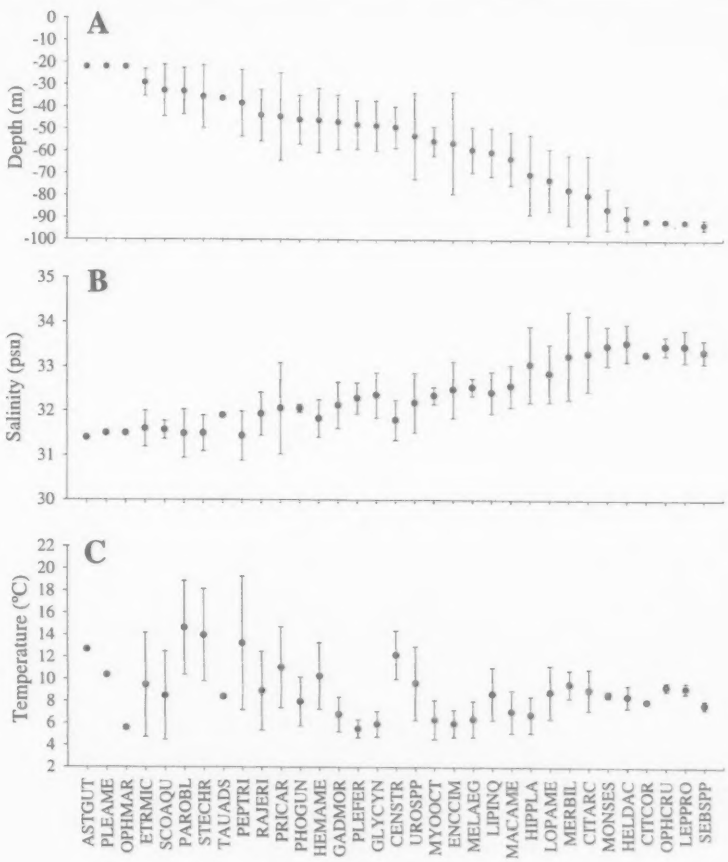

Figure 3

Weighted mean (**A**) depth, (**B**) salinity, and (**C**) temperature (±1 SD) for all species of age-0 fishes. Species are ordered on the *x*-axis of each plot by their mean depth. See Table 3 for definitions of species codes (at bottom).

warming trend, temperatures decreased by about 1°C per month until they reached their minimum in April. Late winter bottom temperatures in 1997 were not as cold as midshelf temperatures during summer 1996.

Salinity was a more conservative hydrographic variable than temperature. Variation in seasonal bottom salinity was slight; all cruises had a salinity range of approximately 31–35.5 psu. Bottom salinities were lowest inshore near inlets (station 1) and the Hudson River (station 15). The correlation between bottom salinity and bottom depth was high (r=0.87). Higher bottom salinities (>34 psu) offshore showed some fluctuation in their distribution on the outer shelf associated with similar fluctuations in warmer bottom temperatures. These warm, high-salinity bottom waters are representative of slope water intrusions (Churchill, 1985; Flagg et al., 1995).

The total catch of benthic organisms from the trawl varied greatly between stations, but there were generally three major groups or types of trawl samples. Groups were delineated to some extent by the depth at which they were collected. Inner-shelf stations (<40 m) typically included such organisms as the common sea star (*Asterias forbesi*) and fig sponge (*Suberites ficus*). Gammarid amphipods, sand shrimp (*Crangon septemspinosa*), and northern moon snail (*Euspira heros*) constituted much of the rest of the nonfish fauna collected there. Sand dollar (*Echina-*

rachnius parma) and valves of surf clams (*Spisula solidissima*) dominated the midshelf group (40–70 m) of benthic organisms. Shell hash collected midshelf consisted of fine fragments of sand dollar tests and larger pieces of clam valves. Small, recently settled rock crabs (*Cancer borealis*) were found in high numbers (>100 per 5-min trawl) during the summer in this type of sample. Margined sea stars (*Pontaster tenuispinus*) and sea scallops (*Placopecten magellanicus*) made up most of the trawl catch for deeper outer-shelf stations (70–90 m). Young pandalid shrimp (*Pandalus montagui*) constituted a large fraction (30–90%) of samples from these stations in late summer and fall. Larger crustaceans such as American lobsters (*Homarus americanus*) and large rock crabs (*C. borealis*) were occasionally abundant (>10 per 5-min trawl) at these stations. Several were collected during each cruise.

Table 5

Mean density (fish/1000 m^2) of 21 abundant age-0 fishes within each of three depth strata: inner shelf (<40 m), middle shelf (41–70 m), and outer shelf (71–95 m) across all sampling periods. Species are grouped by stratum of their highest abundance; only species from Table 4 are included.

	Inner shelf	Middle shelf	Outer shelf
Inner shelf			
Raja erinacea	1.01	0.81	0.03
Etropus microstomus	0.88	0.01	—
Prionotus carolinus	0.21	0.11	0.01
Paralichthys oblongus	0.12	0.03	—
Enchelyopus cimbrius	0.10	0.06	0.08
Scophthalmus aquosus	0.09	0.01	—
Ophidion marginatum	0.06	—	—
Middle shelf			
Pleuronectes ferrugineus	15.31	49.81	2.25
Liparis inquilinus	0.03	1.94	1.11
Urophycis spp.	0.83	0.84	0.58
Macrozoarces americanus	0.03	0.79	0.69
Gadus morhua	0.16	0.38	0.01
Glyptocephalus cynoglossus	0.02	0.23	0.01
Peprilus triacanthus	0.05	0.19	0.05
Myoxocephalus octodecemspinosus	—	0.11	0.01
Centropristis striata	0.04	0.10	—
Outer shelf			
Merluccius bilinearis	0.40	5.42	14.69
Citharichthys arctifrons	0.29	0.64	5.63
Lepophidium profundorum	—	—	0.68
Ophichthus cruentifer	—	—	0.51
Helicolenus dactylopterus	—	—	0.14

Other groups of macrobenthos were ubiquitous. Hermit crabs and cancer crabs of intermediate sizes were found throughout the stations sampled. Although they dominated the midshelf, sand dollars were also collected at inner-shelf stations. Other large benthic fauna such as horseshoe crabs (*Limulus polyphemus*) and spider crabs (*Libinia emarginata*) were collected, but these collections were sporadic.

Species assemblages and environmental variables

Four canonical axes, each representing a linear combination of the environmental data, were calculated from the data set. These four axes together accounted for 36.7% of the variation in species abundance and 82.1% of the cumulative variation, in relation to the total variation explained by the environmental variables. A summary of eigenvalues and the variance accounted for by each axis is given in Table 6A. The variance explained by the entire ordination, as well as the first axis, was more significant than expected by chance, as calculated by a Monte Carlo permutation test (n=99 iterations, P=0.01 for both tests).

Interpretation of the relationships between environmental variables and the CCA axes involves determining which variables are most correlated to the axes. One intuitive and effective method of accomplishing this is to examine an ordination plot of environmental variables (Fig. 9). Environmental variables with large components along a CCA axis have high correlations to that axis. However, the results of the ordination with 25 environmental variables were difficult to interpret because of inherent covariability of variables with one another (Fig. 9, A–B).

Forward selection of environmental variables resulted in selection of five environmental variables (bottom temperature, depth, the relative abundance of scallops, longitude, and the relative abundance of margined sea stars). Eigenvalues of the forward selection of these five variables (Table 6B) are predictably lower than for the CCA with all 25 variables included (Table 6A). However, 59% of the variability explained with all 25 variables included was explained by these five environmental variables alone. Temperature represented 23% of the entire variability explained by the environmental data, depth contributed 18%, scallop abundance explained 8%, and longitude and margined sea star abundance combined explain an additional 5%. The remaining variables, which did not add significantly to the resulting explanation, each explained less than 4% of the variation. Because only five variables were selected, the four synthetic gradients (CCA axes) are each highly correlated with one of the environmental variables (Table 6A). Thus, to some extent each axis

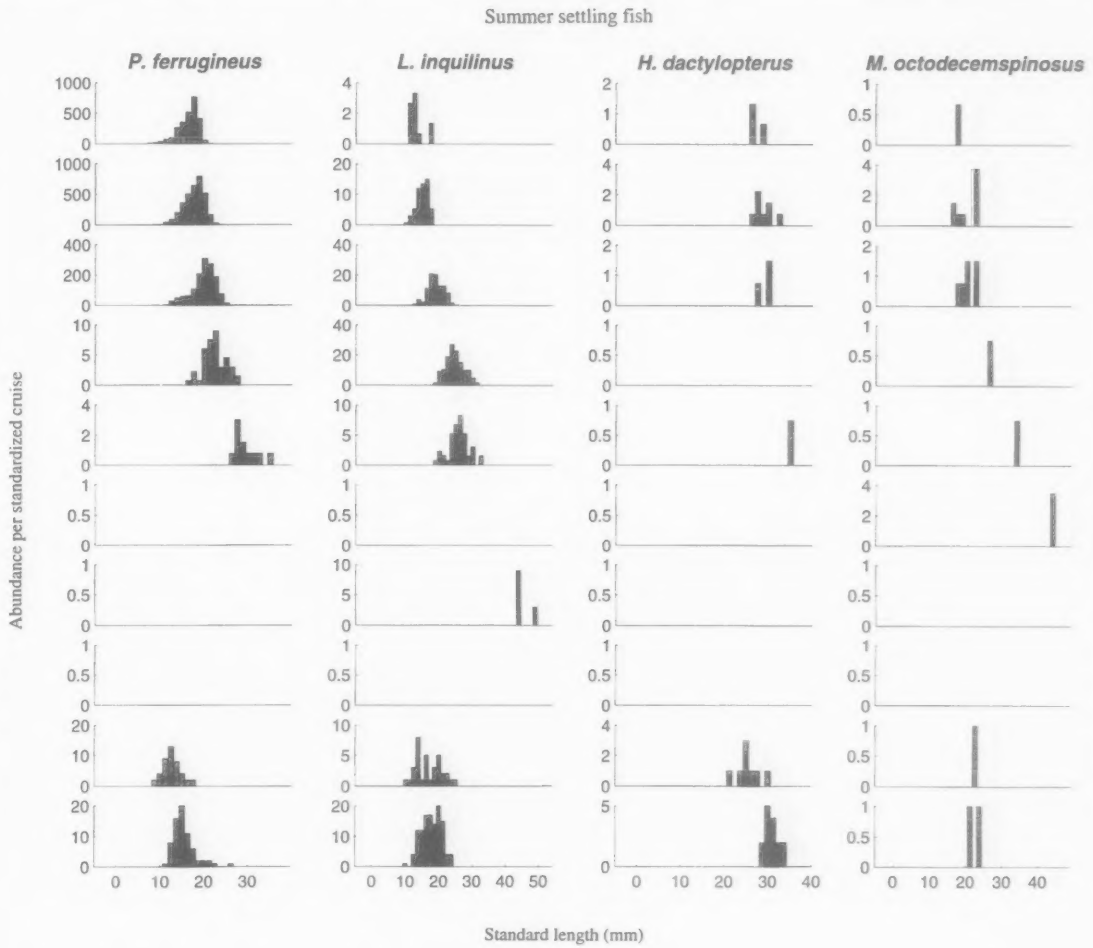

Figure 4

Length frequency by cruise of four species (*Pleuronectes ferrugineus*, *Liparis inquilinus*, *Helicolenus dactylopterus*, and *Myoxocephalus octodecemspinosus*) that settled to the shelf between early summer and midsummer. Plots for each species are ordered from top to bottom according to the order of the 10 cruises (see Table 1 for cruise dates). Abundance on each cruise was standardized to the number of fish collected during 63 5-min tows (21 stations with 3 tows each).

is a proxy for the environmental variable to which it is highly correlated.

Examination of species-environment biplots revealed relationships between environmental variables and species distributions. The species-environment biplot for the first two axes represents the majority of the species-environment relationships that resulted from the partial CCA (Fig. 9C). The importance of depth and temperature to these two canonical axes (see Table 7) indicates that temperature and depth are the two most important determinants of the species data. Inner-shelf species such as *Stenotomus chrysops*, *Paralichthys oblongus*, *Centropristis striata*, and *Etropus microstomus* are located near the upper right-hand quadrant of the ordination (positive values for both of the first CCA axes). Outer-shelf species, such as the rockfishes, *Sebastes* spp., and *Helicolenus dactylopterus* and the snake and cusk eels (*Ophichthus cruentifer* and *L. profundorum)* are found in the lower right-hand quadrant (positive CCA 1 and negative CCA 2). Species from the middle shelf, particularly *Pleuronectes ferrugineus* and *Liparis inquilinus*, are located near the origin of the ordination diagram.

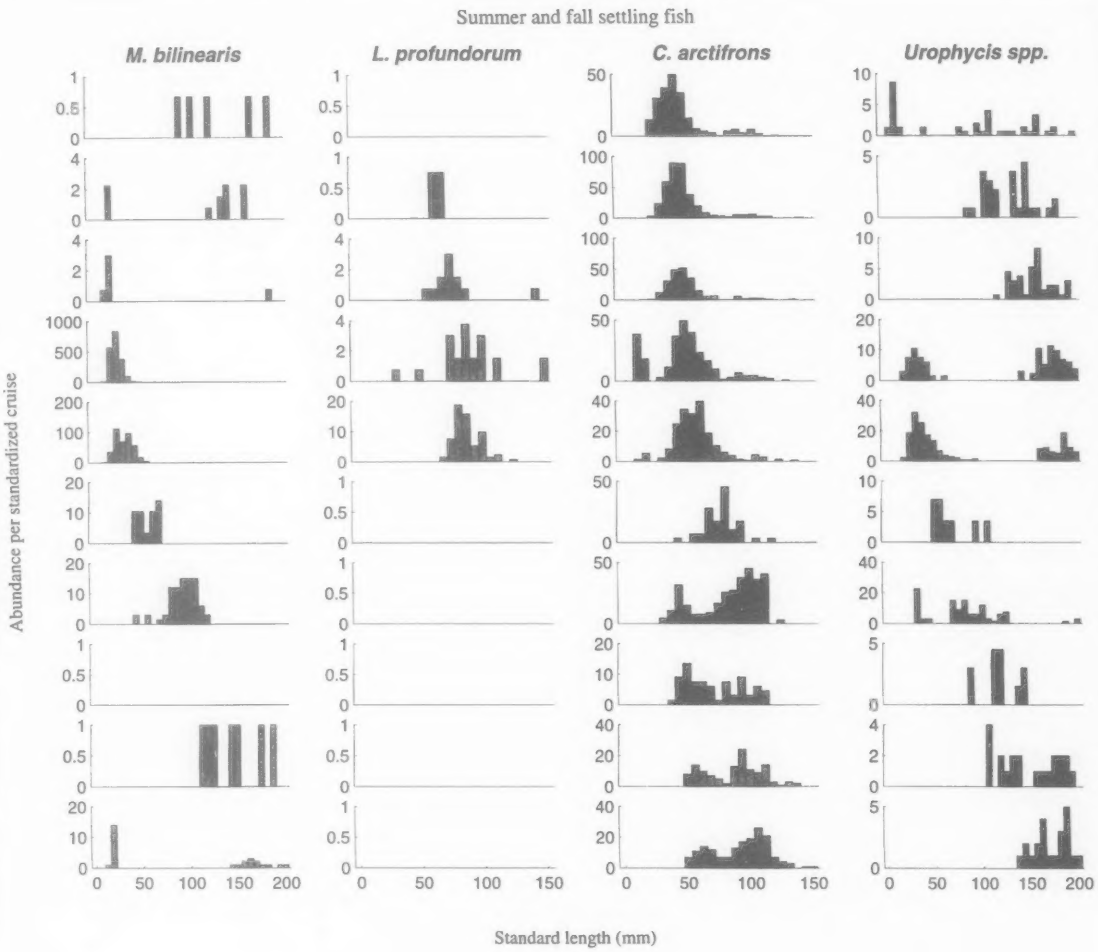

Figure 5

Length frequencies by cruise of four species (*Merluccius bilinearis, Lepophidium profundorum, Citharichthys arctifrons*, and *Urophycis* spp.) that settled to the shelf between late summer and midfall. Plots for each species are ordered from top to bottom according to the order of the 10 cruises (see Table 1 for cruise dates). Abundance on each cruise was standardized to the number of fish collected during 63 5-min tows (21 stations with 3 tows each).

The origin represents the mean for each environmental variable, and those means are weighted by species abundance. Because of the numerical dominance of *P. ferrugineus* in the samples, its mean temperature and depth of collection is driving the location of the origin. Consequently, the location of *P. ferrugineus* is just slightly left of the origin. The mean depth distribution of *P. ferrugineus*, however, was midshelf and thus its abundance did not skew the species distributions about the second axis, CCA 2.

One of the largest effects of the forward selection of variables is that the third and fourth CCA axes (Fig. 9D) now have definite environmental correlates. When 25 variables were used during full CCA, no one variable stood out as a clear contributor to the variation along these axes (Fig. 9B). With the abundance of scallops being the important environmental gradient describing the third CCA axis, one important species association is made clear: the abundances of juvenile inquiline snailfish and scallops. The fourth CCA axis, representative of longitude, accounted for only 5% of the total environmental explanation of the species patterns. This pattern might be considered as the subtle variation among the three transects (west, central, and east).

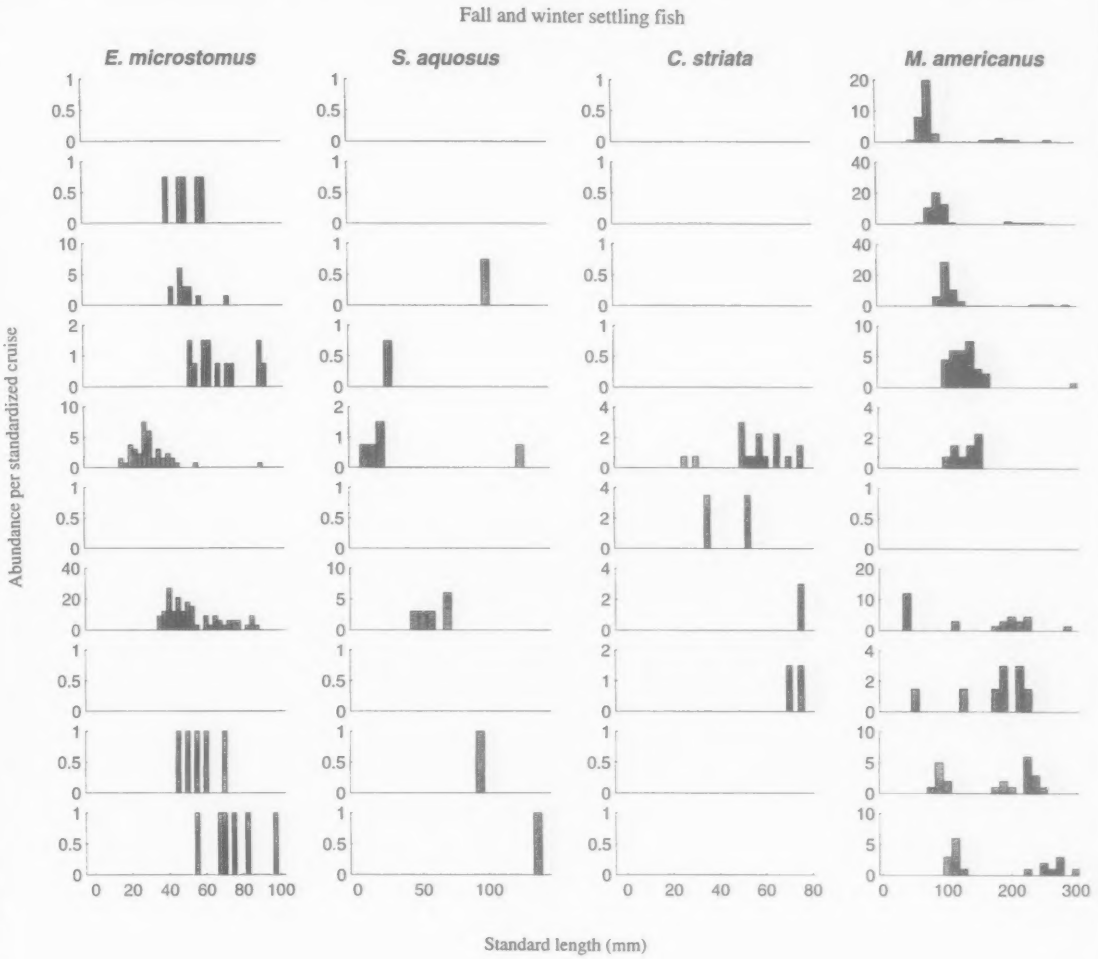

Figure 6

Length frequencies by cruise of four species (*Etropus microstomus*, *Scophthalmus aquosus*, *Centropristis striata*, and *Macrozoarces americanus*) that settled to the shelf between midfall and winter. Plots for each species are ordered from top to bottom according to the order of the 10 cruises (see Table 1 for cruise dates). Abundance on each cruise was standardized to the number of fish collected during 63 5-min tows (21 stations with 3 tows each).

Discussion

Many commercially and ecologically important species of demersal fishes in the NYB settle onto the continental shelf throughout the course of the year. In some cases settlement densities on the shelf may rival those of nearshore and estuarine species. For example, at some stations yellowtail flounder (*P. ferrugineus*) was found to have settlement densities as high as the long-term average for a congeneric estuarine species, plaice (*Pleuronectes platessa*), at approximately one per m^2 (Modin and Phil, 1994). Yet given the large areal extent of *P. ferrugineus* settlement on the shelf, its relative total abundance is potentially much larger. Similarly dense settlement may occur within portions of the shelf environment for other species such as *Merluccius bilinearis*. Even for those species collected in relatively low numbers, the large areas of NYB in which they settle suggests that total numbers are substantial.

Evidence that settlement does occur on the shelf is from two sources. The minimum size of collected

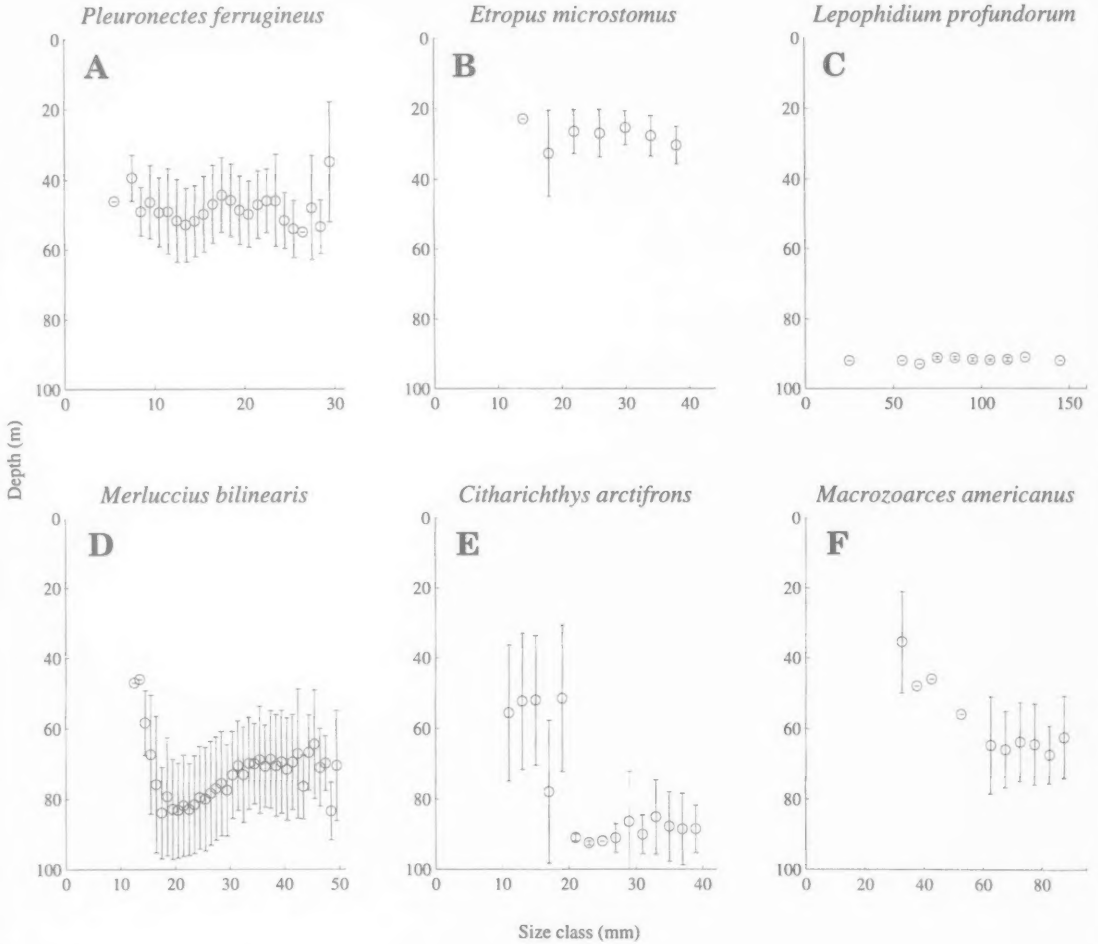

Figure 7

Mean depth (±1 SD) by size class for three species that do not show strong migration (A–C) and three species that do show migration with increased size (D–F).

age-0 fishes (Table 3; Figs. 4–6) were near (and occasionally below) that reported for size at the end of the larval stage (Fahay, 1983). Moreover, although the total contribution of larvae to the trawl catch was low (<2% of numeric abundance), the presence of these specimens in our samples strengthens the assertion that they were collected near first settlement. Most fish larvae collected were metamorphosing or postflexion flounder, particularly *P. ferrugineus*. Larvae from nondemersal fish species known to be present in the water column at this time of year (Kendall and Naplin, 1981; Cowen et al., 1993; Cho, 1996) were not collected by the trawl. Collected larvae, therefore, were likely taken near the bottom rather than during the deployment or recovery of the net.

Compared with settlement areas, nursery areas for groundfishes on the continental shelf are more problematic to define. Gibson (1994) described nursery habitat as being an area where the scope for growth is enhanced for settled juveniles. Scope for growth is a function of habitat quality and quantity, but these parameters, particularly quality, are difficult to measure. Nursery habitats are generally spatially and temporally species-specific, but two or more species may have similar nurseries. Within a single species, nursery and settlement habitats may

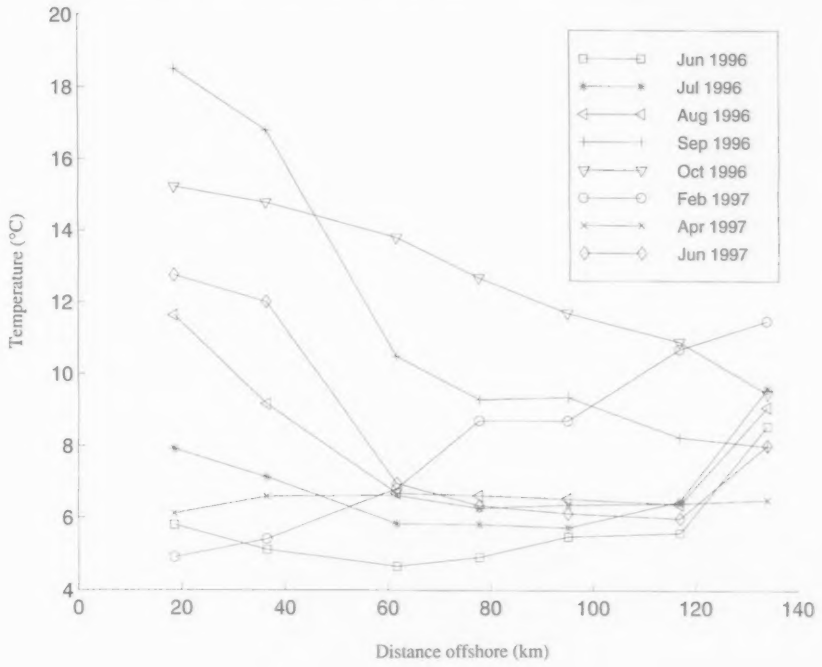

Figure 8

Bottom temperature by distance offshore for the center transect between June 1996 and June 1997.

occur in the same area, include just a slight overlap with one another, or may be entirely separate spatial entities. Although association with settlement habitat is not a given, an area in which postsettled juvenile fishes persist and grow prior to first spawning must be considered to be nursery habitat. Evaluation of nursery habitat quality requires the comparison of growth rates among stations for individual settlement cohorts (Sogard, 1992; Jenkins et al., 1993)—information not presented in our study. For many species, however, growth certainly occurs on the shelf after settlement (Figs. 4–6).

For several of the species collected during this study in the NYB, nursery and settlement habitats are synonymous. For example, *P. ferrugineus*, *E. microstomus*, and *L. profundorum* were limited in their distributions, remaining unchanged from settlement throughout the first year of life (Fig. 7, A–C). Several other species, *M. bilinearis*, *C. arctifrons*, and *M. americanus* (Fig. 7, D–F) showed some movement in depth between settlement and nursery areas, as well as some indication of within-nursery migration across the shelf. Toole et al. (1997) observed similar migration between the settlement and nursery areas of Dover sole (*Microstomus pacificus*) and suggested that such movements were actively pursued by the juveniles, but the ultimate migratory cues remained unclear.

Within the NYB, several environmental gradients describe the spatial and temporal distributions of settlement habitats. Species-environment relationships from the ordination analysis reveal that temperature and depth explain most of the among-species variance in habitat associations. Previous studies of adult fishes in the NYB have shown the importance of temperature and depth in describing species assemblages (Colvocoresses and Musick, 1984). This is in contrast to the west coast of the United States, where fish habitat associations have been studies extensively for rockfish (*Sebastes* spp.) and to a lesser extent for flatfish. There, for many species, more importance has been attributed to geological features such as sediment size and bathymetric heterogeneity (Pearcy, 1978; Stein et al., 1992; Adams et al., 1995). Considering the higher level of geological diversity on the West Coast than in the NYB, this conclusion is not surprising.

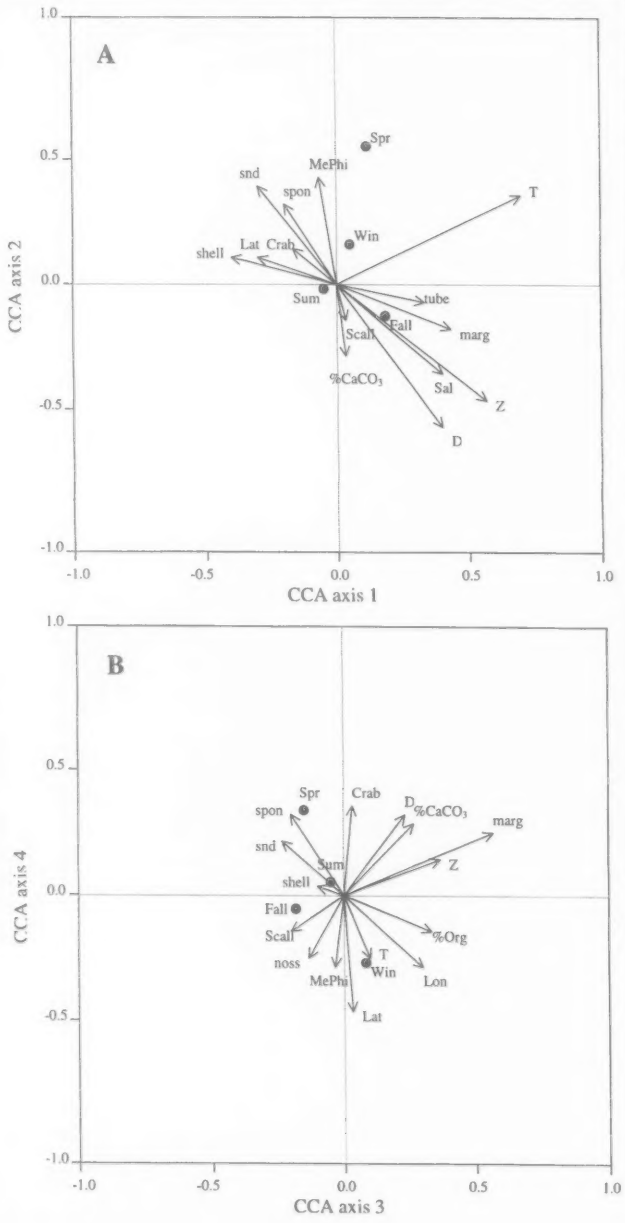

Figure 9

Ordination of the 25 environmental variables with (**A**) the first two and (**B**) the third and fourth canonical axes. Seasonal variables (Spring, Summer, Fall, and Winter) are plotted as points, all other variables as vectors. Environmental variable codes are explicated in Table 2. Species-environment biplots are given in (**C**) for the first two axes and in (**D**) for the third and fourth axes of the partial CCA. Species codes are defined in Table 3. The relative rank of a species with respect to an environmental variable can be determined by extending a line perpendicular from the environmental vector to the species.

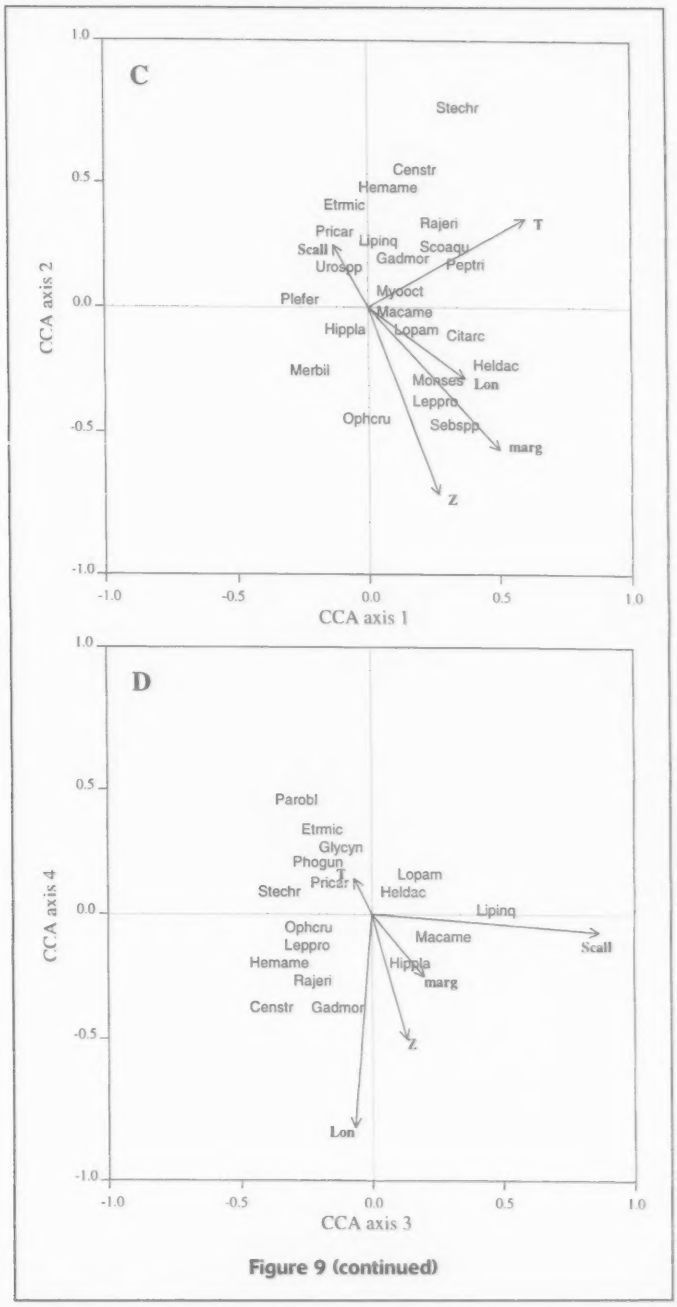

Figure 9 (continued)

Other variables such as salinity, the abundance of benthic organisms, mean sediment size, as well as the proximate location of other essential habitats (e.g. estuaries), were correlated to distance offshore and depth and are thus possible contributors to observed habitat preferences. It is doubtful that a species would prefer habitat based on distance offshore or depth alone because both variables are spatial indices and as such, they can have no direct effect on habitat quality. The indirect effects of these spatial variables on habitat quality may be associated with how they correlate with the distribution of

Table 6

(**A**) Results from the CCA including all 25 environmental variables. The sum of all canonical eigenvalues (2.187) was 44.7% of the total inertia (4.891). (**B**) Results of the CCA with forward selection (5 environmental variables) and season treated as a covariable. The sum of the unconstrained eigenvalues (3.825) is 78.2% of the total inertia, indicating that the seasonal component contributed 21.8% of the variance. The sum of all canonical eigenvalues (0.636) is 16% that of the sum of all unconstrained eigenvalues and 13% of total inertia. See text for explanation of terms.

	Axes				
	1	2	3	4	Total inertia
A					
Eigenvalues	0.809	0.464	0.284	0.238	4.891
Species-environment correlations	0.961	0.873	0.704	0.691	
Cumulative percentage variance					
of species data	16.5	26.0	31.9	36.7	
of species-environment relation	37.0	58.2	71.2	82.1	
Sum of all unconstrained eigenvalues					4.891
Sum of all canonical eigenvalues					2.187
B					
Eigenvalues	0.269	0.230	0.078	0.047	4.891
Species-environment correlations	0.721	0.735	0.518	0.365	
Cumulative percentage variance					
of species data	7.0	13.0	15.1	16.3	
of species-environment relation	42.3	78.5	90.8	98.3	
Sum of all unconstrained eigenvalues (after fitting covariables)					3.825
Sum of all canonical eigenvalues (after fitting covariables)					0.636

important factors or combinations of factors, or with both. For example, age-0 inquiline snailfish (*L. inquilinus*) are found almost exclusively in association with the presence of sea scallops, with which they have their inquiline relationship (Able and Musick, 1976). The distribution of these fishes on the mid to outer shelf is therefore not necessarily directly affected by any physiological response to depth or distance offshore; rather, they are probably most affected by their dependence on sea scallops for shelter. The distribution of scallops in turn may be affected by such variables as temperature, salinity, circulation pattern, and the production of phytoplankton (Stewart and Arnold, 1994). Temperature, however, may have a direct effect on the quality of habitat for a given species because many physiological functions such as respiration, metabolism, and growth are directly controlled by changes in temperature (Neill et al., 1994).

Temperature and depth not only seem to provide the best description of the overall variance within this data set, but, along with salinity and time of year, form the basis for the hydrographic description of the NYB. In general, the bottom waters of the NYB can be broken down by location into three regions: inner-shelf waters, middle shelf (cold pool), and outer shelf. This breakdown of the continental shelf for the NYB is consistent with that of other shelf systems (McRoy et al., 1986; Werner et al., 1997). During the summer, waters overlaying these regions of the shelf are divided by two frontal features: the inner front, dividing the inner and middle

Table 7

Weighted correlations between the five environmental variables selected during the CCA with forward selection and the axes of from the analysis. Numbers in bold indicate the strongest correlation for each of the four axes. See Table 2 for meaning of environmental variable codes

Axis	Temp	Z	Scall	Long	Marg
CCA 1	0.8262	0.4257	–0.0796	0.3723	0.6508
CCA 2	0.4932	–0.7895	0.1636	–0.2067	–0.5973
CCA 3	–0.0819	0.1485	0.9711	–0.0620	0.1028
CCA 4	0.0863	–0.3690	–0.1399	–0.8822	–0.0934

shelves, and the middle front, dividing the middle and outer shelves. A third front, the shelf-break front, separates the outer shelf from the continental slope (McRoy et al., 1986). These three hydrographic features can be utilized as an effective means for describing the general nursery areas for groundfishes within the NYB.

Differences in environmental conditions occur not only along the shelf, but also temporally. Patterns in surficial sediments and bathymetry change on the order of hundreds to thousands of years. These characteristics are relatively stable in their distributions on the shelf. Other factors such as benthic faunal distributions may change from year to year but should remain unvarying during the first year of a fish's life. Hydrographic variables and the presence of ephemeral predator and prey species, however, may change on a seasonal or even daily basis. The scale and extent to which these variables change are important in understanding the nature of the settlement and nursery areas.

The turnover of the cold pool in the fall represents an abrupt change in bottom temperature that subsequently may have biological significance. In our study, higher mean catch of age-0 *P. ferrugineus* corresponded to the distribution of the cold pool, and catch dropped dramatically in the fall as bottom temperature increased during cold pool turnover. For a species with a preference for low temperatures, increased temperature may be a density-independent source of mortality. In at least one case, an increase in mortality of larval *P. ferrugineus* on Georges Bank has been associated with an increase in temperature from a warm-core ring (Colton, 1959). Although such abrupt temperature fluctuations at greater depths are not common, they may occasionally occur near the edge of the shelf in some areas, perhaps associated with Gulf Stream meanders (Able et al., 1993). Able et al. (1993) found a temperature increase of 6°C over a two-day period, and they suggested that such temperature fluctuations may cause a cessation of feeding for tilefish, *Lopholatilus chamaeleonticeps*. In contrast, some species may benefit from increased bottom temperatures associated with turnover. For example, in our study, settlement of *M. bilinearis* did not peak until after turnover, when bottom temperature was greater than 9°C. Likewise, spawning distribution for Atlantic croaker (*Micropogonias undulatus*) in the southern portions of the MAB has a positive association with the presence and location of warmer waters coincident with the cold pool turnover (Norcross and Austin, 1988).

This study and another from Georges Bank (Frank et al., 1992) indicate that the distribution of *P. ferrugineus* corresponds well with bottom temperature. The presence of *P. ferrugineus* is negatively correlated with bottom temperature in the NYB, with highest abundance in the coldest waters (<8°C). However, on the Grand Banks the correlation with temperature was positive, with most fish collected above the warmest bottom waters (>3°C). Juveniles from the Grand Banks, however, were collected off the bottom, and highest abundances were found just below the thermocline, in waters closer to 6°C. Temperatures between 4° and 8°C have been noted as the preferred temperatures for older *P. ferrugineus* (Scott, 1982; Ross and Nelson, 1992; Walsh, 1992), and the evidence from these studies suggests that this may be true for age-0 *P. ferrugineus* as well.

Besides *P. ferrugineus*, several other boreal species, American plaice (*Hippoglossoides platessoides*), haddock (*Melanogrammus aeglefinus*), and Atlantic cod (*Gadus morhua*), were collected in our samples from the cold pool. Although not as numerically dominant as *P. ferrugineus*, the presence of these species this far down the coast suggests that the cold pool may act as a temporary refuge from the warmer waters normally associated with lower latitude. Metapopulations of such species, wherein local populations occupy small areas of suitable habitat outside the main population, are a potential confounding factor to the management of these species (Bailey, 1997). In the extreme, these juveniles may not be viable additions to the population if environmental conditions are not suitable for them to reach maturity. Just as warm-core eddies and eddy streamers may bring expatriates into this area from the south in the summer (Hare and Cowen, 1991), the cold pool may allow boreal species to temporarily extend their range southward during the summer. It is possible that conditions may occasionally enhance survival in such potentially marginal habitats to the extent that success of the population year class is facilitated.

In our study, we used trawl data to describe the general distribution and large-scale habitat associations of age-0 fishes. The resolution obtainable from trawl data is limited to the area swept (about 700 m^2). Such resolution is sufficient to determine differences between stations that are 10 km apart but not to deterine the heterogeneity in habitat within a station. *In situ* methods, including the use of manned submersibles, are required for these types of small-scale studies (see Auster et al., 1991, 1997; Adams et al., 1995). One other drawback of using a trawl to quantify fish abundance is that trawls are inherently poor at collecting all fishes present in a given area; gear selection is size- and species-specific. However, if gear selection is constant between sites, trawl samples should be comparable (Kuipers et al., 1992). The

relative efficiency of our 2-m beam trawl on offshore nursery areas for juvenile flatfish and other demersal species was unknown, although it was likely that our trawl was relatively inefficient but consistent in collecting demersal fishes. Consistent inefficiency among trawl samples would not affect the general patterns that we found but could have led to underestimation of abundances.

For a trawl to be efficient, demersal fishes must remain near the bottom. Recently settled juveniles of some species, such as *M. bilinearis*, have been shown to make nightly excursions away from the bottom to feed (Fahay, 1974). For a fusiform juvenile like *M. bilinearis* this ability is not surprising. However, the pleuronectiform *P. ferrugineus* has been collected well off the bottom on the Grand Banks, not only as larvae but also as juveniles (Frank et al., 1992). These "pelagic juveniles" were of the same size classes (10–34 mm) as the postmetamorphic flounder we collected on the bottom with a 2-m beam trawl in our study. In extensive studies in the MAB with comparable or larger midwater sampling gear, few *P. ferrugineus* larger than 15 mm were collected (Morse, 1989; Cowen et al., 1993). For the NYB, however, juvenile *P. ferrugineus* do not appear to exhibit such trawl-avoiding behavior. This regional difference in vertical distribution of >20-mm postmetamorphic young may be due either to differences in the temperature structure of the water column (i.e. depth of optimal temperature, as discussed above) or to some other unknown latitudinal difference in developmental rate, settlement size, or behavior (Miller et al., 1991; Fuiman and Higgs, 1997; Osse and Boogaart, 1997).

In summary, age-0 demersal fishes utilize the continental shelf of the NYB as both settlement and nursery habitat. According to our findings, the shelf of the NYB can be divided into three broad nursery areas (inner, middle, and outer shelves) and can be described by species assemblage as well as by hydrography. There is a need for more research concerning the quality of habitats for age-0 fishes in the NYB. Information on smaller-scale variation in habitat for age-0 fishes may be just as revealing as that for larger macrobenthos (see Auster et al., 1991). Manned submersibles may be used to determine such small-scale habitat associations, even for small juveniles. Differences in growth rates of given species among habitats should also shed light on habitat quality within the NYB. Nursery habitats play an important role in the life history of marine fishes, and knowledge gained about the distribution and quality of these areas for commercially important offshore species should help to improve management of these areas..

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Assemblage of deep-sea sharks on Chatham Rise, New Zealand

Bradley M. Wetherbee
Zoology Department
2538 The Mall
University of Hawaii
Honolulu, Hawaii 96822
Present address: Northeast Fisheries Science Center
National Marine Fisheries Service, NOAA
28 Tarzwell Dr., Narragansett, Rhode Island 02882
E-mail address: brad.wetherbee@noaa.gov

Abstract.—The distribution and abundance of deep-sea sharks on Chatham Rise, New Zealand, are described. Sharks were collected as bycatch in two deep-water trawl fisheries at a total of 390 stations, which ranged in depth from 740 to 1503 m. Sixteen species of shark were caught; *Deania calcea*, *Centroscymnus crepidater*, *Etmopterus granulosus*, and *Centroscymnus owstoni* accounted for the largest portion of the shark catch. Species that would provide the highest yield of commercially important liver lipids were not abundant in trawls. All sharks combined formed only 4.2% of overall biomass captured in trawls. Depth is a major determinant of the composition of the shark assemblage; both density of sharks (kg/km^2) and species diversity were inversely proportional to depth. Distributional patterns of the shark community varied with location on Chatham Rise, and species composition of the shark catch varied with the species of teleost targeted in deep-water trawls.

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Sharks are common bycatch in deep water fisheries around the world, forming as much as 50% of the catch in deep-sea trawls in areas such as New Zealand and Australia (Deprez et al., 1990; Clark and King[1]). Most sharks captured in the New Zealand and Australian deep-water fisheries are dead by the time they are brought to the surface and are discarded, but some sharks are retained for their liver oil. In Japan and Australia, several species of deep-sea shark in the family Squalidae are targeted in fisheries and their liver oil is utilized. Although the short-term potential of fisheries directed towards deep-sea sharks has been investigated for a few species (Summers, 1987; Davenport and Deprez, 1989), little information on even basic biology is available for the species captured in these fisheries. Thus, the effects that deep-water fisheries have on shark populations that are either targeted directly or caught incidentally are unknown. Information on abundance, distribution, community structure, reproduction, and age and growth of deep-sea sharks would improve understanding of these effects.

Shark liver oil contains commercially important lipids, such as squalene and diacyl glycerol ether, which are used in cosmetic, pharmaceutical, and other industries (Deprez et al., 1990; Bakes and Nichols, 1995). The lipid composition of liver oil is quite variable among and within species, and consequently the most desirable sharks are those individuals and species that have the highest potential as a source for these valuable lipids (Davenport and Deprez, 1989; Bakes and Nichols, 1995). Therefore, understanding the distribution and abundance of different species of deep-sea shark, in conjunction with knowledge of the lipid composition of their liver oil, is important for optimal use of these resources.

Some deep-sea sharks prey upon commercially important teleosts (Clark et al., 1989; Clark and King[1]), but the impacts of shark predation on fish populations in terms of the overall economic impact on the fishery are unknown. Diet varies considerably among even closely related species of deep-sea shark (Compagno et al., 1991; Ebert et al., 1992), and the level of predation on commercially important species of teleost by sharks also varies among species (author's unpubl. data). Information on the distribution and abundance of deep-sea sharks, in conjunction with knowl-

[1] Clark, M. R., and K. J. King. 1989. Deep water fish resources off the North Island, New Zealand: results of a trawl survey, May 1985 to June 1986. New Zealand Fisheries Technical Report 11, 56 p. MAF Fisheries Research Center, P.O. Box 297, Wellington, New Zealand.

edge of their feeding habits, would improve our understanding of interactions between sharks and commercially important teleosts.

A variety of species of shark inhabit the deep water off New Zealand, where they form part of the bycatch of deep-sea fisheries that target teleosts such as orange roughy (*Hoplostethus atlanticus*) and smooth oreo (*Pseudocyttus maculatus*) (Clark and Tracey, 1994; Clark and King[1]). Access to this bycatch provided an opportunity to examine a multispecies complex of sharks, which might be termed an assemblage—"a group of co-occurring populations—not necessarily interacting" as defined by Crowder (1990). The purpose of this study was to investigate the abundance and distribution of sharks on Chatham Rise to increase understanding of the effects of fishing on shark populations, the potential of shark fisheries and utilization of bycatch, and interactions between sharks and commercially important teleosts.

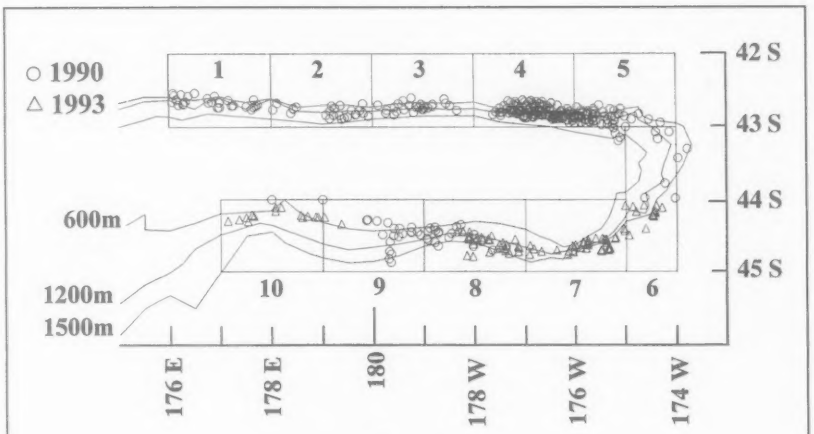

Figure 1

Map of Chatham Rise, New Zealand, showing depth contour lines and locations of trawls in a 1990 orange roughy survey and a 1993 smooth oreo survey. Trawls were grouped into 10 areas on the basis of major latitude and longitude meridians.

Materials and methods

Data for this study were collected from deep-water bottom trawls during two cruises conducted by the Ministry of Agriculture and Fisheries on Chatham Rise, New Zealand (Fig. 1). The first survey (15 June to 5 August 1990) consisted of 281 trawls for orange roughy (*H. atlanticus*) and was conducted primarily on the north of Chatham Rise from the FV *Cordella*. The second survey (24 October to 9 November 1993) consisted of 109 trawls for smooth oreo (*P. maculatus*), primarily on the south of Chatham Rise from the RV *Tangaroa*. Fishing during both surveys was conducted at depths of 740–1503 m throughout the day and night (Fig. 2).

Each survey consisted of a stratified random trawl design intended to provide relative biomass estimates and to illustrate patterns of distribution of deep-water species on Chatham Rise. Six-panel bottom otter-trawls with cut-away lower wings were used in each survey. The door-spread was 75 m for orange roughy trawls and 119 m for smooth oreo trawls, and distance between the net wings for both trawls was approximately 26 m. Codend mesh sizes were 110 mm for orange roughy trawls and 100 mm for smooth oreo trawls. Towing speed for both vessels was approximately 3.0 kn. Orange roughy trawls were roughly 1 h in duration, and smooth oreo trawls ranged from several minutes to 45 min. For density estimates (kg shark/km^2) it was assumed that herding by, and escape from, nets were minimal, and that trawls sampled different species of shark with equal effort.

For each trawl, the catch was sorted into bins by species, and the total weight of each species caught at each station was recorded. Latitude, longitude, water temperature, minimum and maximum depth of fishing, towing speed, and start and end time were also recorded for each trawl. When the author was present on the research vessel (at all times other than from 15 June to 10 July 1990), all individuals of each species of shark were weighed and measured, except when large numbers of sharks were caught and a lack of time prevented examination of every shark. Because of size varied among species, an estimate of the total number of individuals captured in all fishing was derived by using the average weight for each species. Because there were differences in fishing methods (net characteristics, trawl duration) and time (season, year) between the two surveys, catch data from surveys were examined separately. When possible, comparisons were made between common areas fished during both surveys. For comparison of the composition of the shark community at different locations on Chatham Rise, ten areas were designated based on major latitude and longitude meridians (Fig. 1).

Consideration of sharks as an assemblage, which is separate from the rest of Chatham Rise community, is an artificial division. However, because the primary interest of this study was to describe the abundance and distribution of the sharks on Chatham Rise, several ecological indices were employed to compare different locations, depths, and species of shark. Abundance was expressed as density (kg shark/km^2) and was calculated for each species within each trawl based on the total weight of sharks caught, net width, towing speed, and trawl duration. Three features of distribution were examined for sharks: diversity, similarity, and randomness. Diversity was expressed as the number of species of sharks per trawl (Stephens et al. 1984; Garcia et al. 1998). The Bray-Curtis similarity index was used for comparisons among the ten areas on Chatham Rise, depth intervals, and between the two surveys:

$$S = 1 - \left(\sum_{i=1}^{s} |Y_{ij} - Y_{ik}| / \sum_{i=1}^{s} (Y_{ij} + Y_{ik}) \right),$$

where Y_{ij} = score for i^{th} species in the j^{th} sample; and
Y_{ik} = score for the i^{th} species in the k^{th} sample (Field et al. 1982; Sedberry and Van Dolah 1984).

This index ranges from 0 (no species in common) to 1 (identical species in each sample). Morisita's index of dispersion (I_d) was calculated for each species of shark as an indicator of the randomness of their distributions:

$$I_d = n\left(\sum X^2 - N / N(N-1) \right),$$

where n = number of plots;
N = total number of individuals counted in all n plots; and
ΣX^2 = squares of the number of individuals per plot summed over all plots.

If the dispersion is random, then I_d = 1.0; if perfectly uniform, I_d = 0; and if maximally aggregated (all individuals in the same trawl), I_d = the number of trawls (Brower and Zar, 1984). To minimize the dominant effect of anomalous catches, each value of shark density (kg/km^2) was converted to $\ln(x+1)$ prior to calculation of these ecological indices (Field et al., 1982; Bianchi, 1991) and comparisons of means were made by using either a two-tailed t-test or one-way ANOVA.

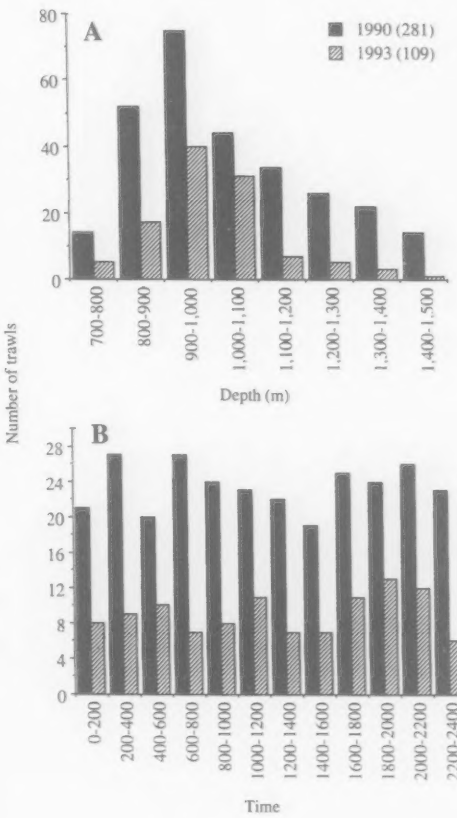

Figure 2

Number of trawls in a orange roughy survey (1990) and in a smooth oreo survey (1993) on Chatham Rise, New Zealand, at various intervals of (**A**) depth and (**B**) time of day. Numbers in parentheses are total trawls for each survey.

Results

Seven species of shark belonging to the family Squalidae and five undescribed species of catsharks in the genus *Apristurus* (family Scyliorhinidae) were regularly captured in deep-water trawls (Table 1). For the purpose of this study, all sharks of the genus *Apristurus* were treated as a single group. Several other species of shark were captured (*Chlamydoselachus anguineus*, *Centroscymnus coelolepis*, *Scymnodon squamulosus*, *Etmopterus lucifer*), but only one or two individuals of each species were captured and these are not discussed further. Although the composition of the catch varied throughout the day, there was no apparent correlation between density

Table 1

Catch data for eight taxa of sharks from trawls on Chatham Rise, New Zealand, 1990 (June–August, 281 trawls, depth range = 743–1503 m) and 1993 (November, 109 trawls, depth range = 780–1463 m). D.c. = *Deania calcea*, C.c. = *Centroscymnus crepidater*, E.g. = *Etmopterus granulosus*, C.o. = *Centroscymnus owstoni*, C.s. = *Centrophorus squamosus*, A.s. = *Apristurus* spp., S.p. = *Scymnodon plunketi*, D.l. = *Dalatias licha*. (SD = standard deviation).

	kg shark caught					% of total biomass			% of trawls			Avg. density (kg/km^2)			
Species	1990	1993	Total	Avg. wt. (kg)	Estimated no. of individuals	1990	1993	Total	1990	1993	Total	1990	1993	Total	SD
D.c.	6721.6	3618.7	10340.3	2.757	3750	1.38	2.10	1.57	65.7	50.5	61.5	172.5	343.5	220.3	590.3
C.c.	6058.0	351.3	6409.3	1.980	3237	1.25	0.20	0.97	58.6	39.2	53.2	151.2	61.8	126.2	266.5
E.g.	3072.2	2881.4	5953.6	1.416	4203	0.63	1.67	0.90	75.7	88.8	79.3	93.5	960.8	335.9	1624.5
C.o.	3731.9	25.5	3757.4	5.023	748	0.77	0.01	0.58	46.8	3.7	34.9	88.9	2.3	64.7	205.8
C.s.	181.0	81.7	262.7	8.209	32	0.04	0.05	0.04	7.1	7.5	7.2	4.7	33.1	12.6	115.2
A.s.	144.1	89.9	234.0	1.245	188	0.03	0.05	0.04	30.7	38.3	32.8	3.5	17.3	7.4	33.3
S.p.	128.7	105.8	234.5	9.770	24	0.03	0.06	0.04	5.0	6.5	5.4	3.0	23.0	8.6	88.9
D.l.	173.1	41.0	214.1	9.309	23	0.04	0.02	0.03	6.8	4.7	6.2	4.3	7.7	5.3	39.9
All sharks	22200.6	9188.3	27405.9		12205	4.15	4.17	4.16	90.3	97.2	92.2	521.8	1449.5	781.1	1786.6

and time of day for all sharks combined (ANOVA, P=0.77). Water temperature decreased linearly with depth (r^2=0.61) and ranged from 5.9° to 9.0°C.

The most abundant shark (by weight) was the shovelnose dogfish (*Deania calcea*), which represented 32.5% of the shark catch in 1990, 50.5% in 1993, and 37.2% overall. The longnose velvet dogfish (*Centroscymnus crepidater*), southern lantern shark (*Etmopterus granulosus*), and Owston's dogfish (*Centroscymnus owstoni*) also formed large proportions of the shark catch (Table 1). The largest catch (by weight) for any species of shark in a single trawl was 850 kg for *D. calcea* (area 5), followed by 441 kg for *C. owstoni* (area 1). Although sharks dominated the catch in some trawls, they formed only 4.2% of total biomass collected in trawls. Even the most abundant species (*D. calcea*) accounted for only 1.6% of total biomass caught (Table 1). The highest estimate for number of individuals of any species of shark captured in a single trawl was 308 for *D. calcea*, followed by 194 for *E. granulosus*.

The most abundant shark in terms of number of individuals captured was *E. granulosus*, which was present in 79.3% of trawls (Table 1). *Deania calcea* and *C. crepidater* were also captured in large numbers and appeared in a high proportion of the trawls. For three of the larger species of squalids (the leafscale gulper shark, *Centrophorus squamosus*; the Plunket shark, *Scymnodon plunketi*; and the kitefin shark, *Dalatias licha*) few individuals were captured, and these species occurred in a low percentage of the trawls (Table 1). Preliminary examination of stomach contents revealed that orange roughy were most common in stomachs of *E. granulosus* and *C. owstoni*, but were also consumed by *C. squamosus* and *D. licha*.

Abundance

There was a significant difference between mean densities (kg/km^2) caught in the two surveys for *E. granulosus*, *C. owstoni*, *C. crepidater*, and *Apristurus* spp., but not for the other species (ANOVA, P<0.01). A comparison of common areas that were fished in both surveys (areas 6, 8, 9, and 10) showed that there was no significant difference between the densities of any of the species for the two surveys (t-test, P>0.01), and data from common areas for the two surveys were therefore combined. In the orange roughy survey, there was a significant difference among areas for densities of all species except *D. licha*, whereas in the oreo survey, significant differences were observed among areas for only two species, *C. crepidater* and *D. calcea* (ANOVA, P<0.05). The highest densities for all sharks combined were recorded at the eastern tip of Chatham Rise, in areas 5 and 6 (1003.4 and 2249.1 kg/km^2), and the lowest were in the southwestern areas 8 and 10 (257.7 and 254.4 kg/km^2).

Composition of the shark catch varied considerably with location fished. In areas on the north of Chatham Rise, closest to New Zealand (areas 1 and 2), the catch was dominated by *C. owstoni* and *C. crepidater*, which accounted for 84% of the shark catch by weight (Fig. 3). On the mid-north of Chatham Rise (area 3), *C. owstoni* and *C. crepidater* still formed the majority of the catch; however *D. calcea* was also abundant and all eight major taxa were recorded. The northeast of Chatham Rise included those areas (4 and 5) where the most trawls were conducted. Here, *C. crepidater* still formed a large

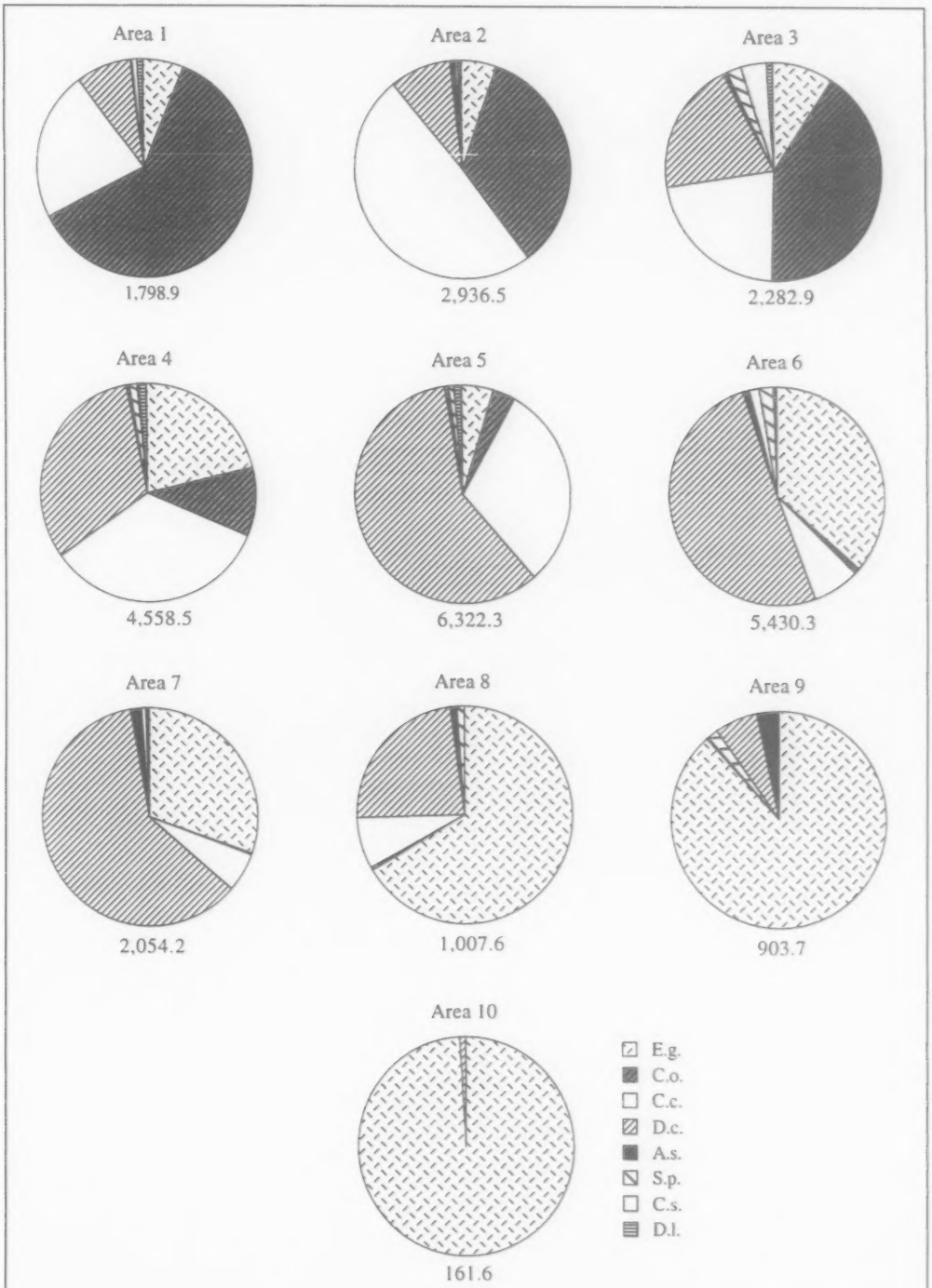

Figure 3

Species composition (as percent of total kg of shark caught) of the shark catch for both surveys combined in each of the 10 areas on Chatham Rise, New Zealand. Numbers below each area are the total weight (kg) of sharks caught in that area. E.g. = *Etmopterus granulosus*; C.o. = *Centroscymnus owstoni*; C.c. = *Centroscymnus crepidater*, D.c. = *Deania calcea*; A.s. = *Apristurus* spp.; S.p. = *Scymnodon plunketi*; C.s. = *Centrophorus squamosus;* and D.l. = *Dalatias licha*.

part of the catch, but the catch of *C. owstoni* declined substantially, and *D. calcea* began to dominate the shark catch. Along the eastern tip and southeast portion of Chatham Rise (areas 6 and 7), *D. calcea* accounted for the highest percentage of the catch, but *E. granulosus* was also prominent, and these two species formed over 85% of the shark catch by weight. *Etmopterus granulosus* dominated catches in areas along the south of Chatham Rise (areas 8–10); the proportion of the total catch increased with proximity to New Zealand (Fig. 3). The three large squalids (*C. squamosus*, *S. plunketi*, *D. licha*) were sporadically caught in areas 1–8, each with a peak density in area 6, but no squalids were recorded from the southwest of Chatham Rise (areas 9 and 10). *Apristurus* spp. were caught in small numbers throughout Chatham Rise, but their presence was more dependent upon depth than location (Fig. 3).

Composition of the catch also varied with depth (Fig. 4) and several natural divisions were apparent. The three large squalids (*C. squamosus*, *S. plunketi*, *D. licha*) appeared to have shallow distributions, and were not captured deeper than 1100 m (with the exception of one *S. plunketi* caught at 1170 m, and one *C. squamosus* at 1201 m). Densities (kg/km^2) of the other species were fairly high at depths of 700–1200 m, although *D. calcea* was most abundant at depths of less than 1000 m, *E. granulosus* peaked at 900–1200 m, and *Apristurus* spp. densities were highest at depths greater than 1000 m (Fig. 4). At depths greater than 1200 m, *Apristurus* spp. were the only sharks regularly caught in the trawls. There was a significant difference among three depth intervals (700–1000 m, 1000–1300 m, and 1300+ m) for mean densities (kg/km^2) of each species in the orange roughy survey, but only for the three most abundant species (*D. calcea*, *C. crepidater,* and *E. granulosus*) in the oreo survey (ANOVA, $P<0.05$). Density (kg/km^2) for all sharks combined gradually declined with depth between 700 and 1200 m, but was low at depths greater than 1200 m (Fig. 5).

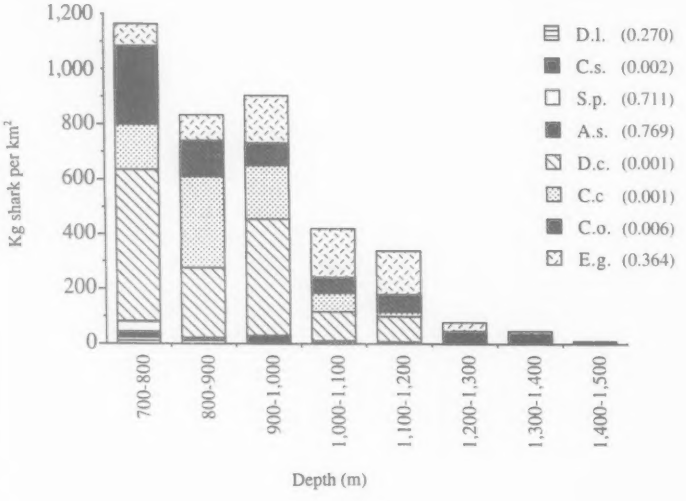

Figure 4

Density (kg/km^2) of sharks collected at all locations on Chatham Rise, New Zealand, at various depth intervals for both surveys combined. For abbreviations of species see Figure 3. For each species, P -values are given in parentheses for ANOVA comparisons of densities among depth intervals.

Distribution

Diversity (number of species per trawl) was significantly higher for the orange roughy survey than for the oreo survey (t-test, $P=0.0003$), but diversity in areas common to both surveys (areas 6, 8, 9, 10) did not differ (t-test, $P=0.14$, 0.77, 0.81, and 0.63 respectively). There was a significant difference among areas for mean diversity values in both surveys (ANOVA, $P<0.01$). Area 3 had the highest mean diversity value (4.2, SD=1.6) and area 10 had the lowest value (1.1, SD=0.9). Diversity differed significantly among the three major depth intervals (700–1000 m, 1000–1300 m, 1300+ m) for the oreo survey (ANOVA, $P=0.007$), but not for the orange roughy survey (ANOVA, $P=0.50$). The total number of species of shark caught in trawls was inversely proportional to depth, and declined by half from a maximum of ten (800–900 m) to only five species at depths of 1200–1300 m (Fig. 5).

The index of similarity (S) between the two surveys was high (0.89), and for each survey there was a high degree of similarity between areas, except for those most distant from each other, particularly between area 10 and other areas (Table 2). Indices of similarity between depth intervals were very similar for both surveys: high (0.80 for the orange roughy survey and 0.81 for the oreo survey) for the two shallowest depth intervals (700–1000 m versus 1000–1300 m), moderate (0.57 and 0.63) for the 1000–1300 m vs 1300+ m intervals, and much lower (0.42 and 0.47) for

700–1000 m versus 1300+ m intervals.

Morisita's index of dispersion (I_d) indicated that all species of sharks tended to be aggregated on Chatham Rise to some degree. The three large squalid sharks were the most aggregated: *S. plunketi* (I_d=104.5), *C. squamosus* (82.4), *D. licha* (55.4), followed by *E. granulosus* (24.3), *Apristurus* spp. (21.2), *C. owstoni* (10.9), *D. calcea* (8.2), and the most randomly distributed was *C. crepidater* (I_d=5.4).

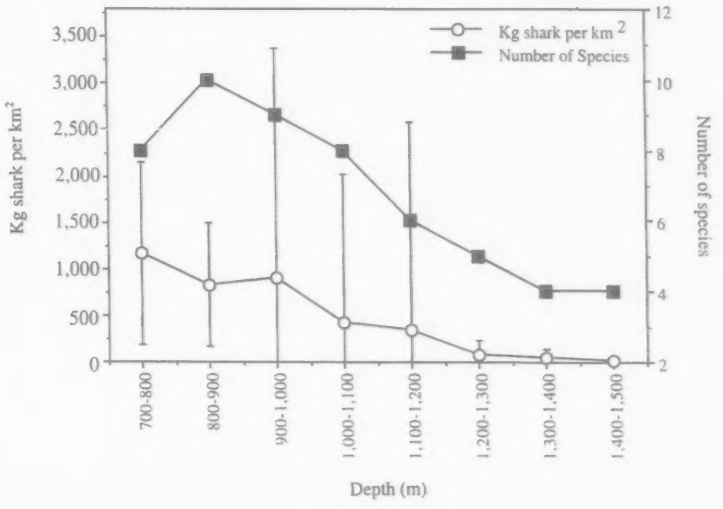

Figure 5

Average density (kg/km² ±SD) of all sharks combined and number of species captured within various depth intervals on Chatham Rise, New Zealand, for both surveys combined. For comparison of mean densities among intervals with ANOVA, P = 0.011, df = 389.

Discussion

Sharks are abundant and widely distributed on Chatham Rise. Even though sharks form a relatively small percentage of the overall catch in deep-water trawls they are frequently caught by the hundreds and occasionally dominate catches. The most abundant shark (by weight) on Chatham Rise, *D. calcea*, was often caught in large numbers, which suggests the presence of large aggregations. However, this species was caught in a high percentage of trawls and was widely distributed on Chatham Rise, which resulted in a fairly low index of dispersion (I_d). *D. calcea* is abundant elsewhere in New Zealand waters, accounting for as much as 70% of the shark catch off the North Island (Clark and King[1]). Kobayashi (1986) reported that *D. calcea* was one of the most common sharks in deep-water catches from Japan as well. The most ubiquitous shark in terms of presence at nearly all depths and locations on Chatham Rise was *E. granulosus*, although this species may have a fairly limited distribution outside of New Zealand and southeast Australia. Tachikawa et al. (1989) synonymized the New Zealand lantern shark, *E. baxteri*, with the widely-distributed southern lantern shark, *E. granulosus*; however, there may be several species within the *E. granulosus* group and *E. baxteri* may well be a valid species (Compagno et al., 1991; Wetherbee, 1996).

Catsharks captured in trawls were keyed out to five undescribed species (A–E) belonging to the genus *Apristurus* (Paulin et al., 1989). That several hundred specimens of these undescribed sharks were collected, underscores the paucity of information on deep-sea sharks. This genus also contains many undescribed species in other locations, such as Australia (Last and Stevens, 1994), and may eventually be recognized as one of the most speciose genera of sharks.

Table 2

Bray-Curtis index of similarity between geographical areas on Chatham Rise, New Zealand for a 1990 orange roughy survey and a 1993 smooth oreo survey. For location of areas see Figure 1.

Orange roughy survey								
Area	2	3	4	5	6	7	9	10
1	0.87	0.84	0.84	0.74	0.69	0.71	0.56	0.38
2		0.89	0.87	0.85	0.78	0.71	0.56	0.36
3			0.84	0.82	0.82	0.68	0.53	0.34
4				0.87	0.85	0.75	0.61	0.42
5					0.89	0.65	0.58	0.36
6						0.72	0.64	0.42
8							0.81	0.60
9								0.71

Oreo survey				
Area	7	8	9	10
6	0.76	0.71	0.35	0.32
7		0.76	0.47	0.37
8			0.57	0.53
9				0.85

Very few individuals of three species of relatively large squalid sharks (*Centrophorus squamosus*, *Scymnodon plunketi*, and *Dalatias licha*) were captured on Chatham Rise. These three species also happen to have liver oil that is high in squalene, or that has a high diacyl glycerol ether to triglycerol ether ratio, and the liver oil of these sharks is thus of high quality for industrial purposes (Bakes and Nichols, 1995; Wetherbee, 1998). Because each of these species formed less than 1% of the total shark catch in all fishing, targeting of any of these species by commercial fisheries on Chatham Rise does not appear to be practical. However, these species have been captured in greater numbers in fishing that was conducted at shallower depths and that targeted different fishes at locations other than Chatham Rise in New Zealand (Clark and King[1]).

The consistency in catch rate, regardless of time of day, indicates that there are few changes in the distribution of the deep-sea shark community on a diurnal basis. However, capture in a trawl may not provide information on activity patterns or feeding periodicity. For example, Kobayashi (1986) found that capture rate of sharks was higher at night than during the day in deep-sea fishing with baited lines. The present study was conducted over a three-month period and does not provide much information on seasonal differences in the distribution of deep-sea sharks. Some studies have suggested that there is continual movement of reproductive groups, or age classes, out of a particular area (Yano, 1991; Wetherbee, 1996). Other studies have maintained that community structure, temperature, and salinity of the deep-sea environment vary little throughout the year (Kobayashi, 1986; Clark and King[1]).

Orange roughy appear to be common prey of two species of sharks (*E. granulosus* and *C. owstoni*) and are also consumed by two of the less common, large squalids (*C. squamosus* and *D. licha*). *Centroscymnus owstoni* may exert the greatest predation pressure on orange roughy populations because both species are found in large numbers on the north of Chatham Rise. An expanded investigation of the feeding habits of sharks would provide more information on the relationship between sharks and commercially important teleosts on Chatham Rise.

Abundance

Differences in abundance of sharks between the orange roughy and oreo surveys appear to be attributable to the location at which fishing was concentrated in each survey. For the oreo survey, fishing was restricted to the south of Chatham Rise, and during the orange roughy survey, fishing was concentrated on the north of Chatham Rise (although trawls were made throughout Chatham Rise). The observation that there were no significant differences between the catches in areas common to both surveys indicates that overall differences between the surveys were probably not due to differences in fishing methods (duration of trawls and net specifications) or time (season or year). The contribution of sharks to total biomass of each survey was also remarkably similar.

The composition of the shark community was dependent upon the area of Chatham Rise that was sampled. Moving from west to east on the north of Chatham Rise, the dominant species in trawls changed from *C. owstoni* to *D. calcea*. Fishing areas at the eastern tip of Chatham Rise were the most diverse. There, the highest densities (kg/km^2) were recorded for six of the eight species of sharks and for all sharks combined. *Etmopterus granulosus* was the most abundant shark on the south of Chatham Rise, completely dominating the catch in the westernmost areas. Variation in the composition of the deep-sea shark community with location has been observed in other areas off New Zealand, and also in Japan and southern Africa (Kobayashi, 1986; King and Clark, 1987; Compagno et al., 1991). In several studies, the community of deep-sea sharks was thought to vary with latitude (Merrett and Marshall, 1981; Nakaya and Shirai, 1992; Yano and Kugai, 1993). The distribution of sharks on Chatham Rise may be influenced by a number of physical and biological factors. However, the lack of information on the deep-sea environment in this area precluded examination of the relationship between shark abundance and either biotic or abiotic factors; in contrast to other studies (Graham and Hastings, 1984; Bianchi, 1991; 1992; Garcia et al., 1998). Large portions of Chatham Rise are as shallow as 500 m, and sharks with fairly deep distributions might not move freely between the north and south slopes. Thus, the patterns of distribution observed for several species of sharks near Chatham Rise may be related to this physical barrier.

In this study, shark abundance also varied with depth. The depth range at which maximum shark density was recorded in the present study (700–800 m) was deeper than that reported by Kobayashi (1986) (300–500 m), and shallower than that found by Yano and Kugai (1993) (1100–1200 m). Depth distributions of sharks caught on Chatham Rise are characterized by several patterns. Some of the larger species were rare at depths greater than 1100 m, which may approximate their maximum depth of occurrence. The density of other species declined abruptly beyond 1200 m, and the proportion of *Apris-*

turus spp. in the catch increased at depths greater than 1200 m. The depths fished in the present study did not appear to reveal the minimum depth of occurrence for any species of shark. Compagno et al. (1991) noted the importance of determining minimum depth of occurrence describing the depth distribution of a particular species.

On Chatham Rise, density (kg/km^2) of all sharks combined was fairly constant up to about 1200 m but dropped drastically beyond this depth. Nakaya and Shirai (1992) observed a similar dramatic decrease in shark density at 500 m, and Merrett and Marshall (1981) at 1000–1100 m. An inverse relation between shark abundance and depth has been described for a number of species in other regions of New Zealand, and in other parts of the world (Merrett and Marshall, 1981; Kobayashi, 1986; King and Clark, 1987; Yano and Kugai, 1993).

Distribution

Diversity (species of shark/trawl) was higher for the orange roughy surveys than for the oreo surveys, but there was no significant difference in mean diversity between areas common to both surveys. These observations support the contention that differences in the shark catch between surveys are related to sampling location, rather than to temporal or methodological differences between surveys. However, there were generally more trawls within each area during the orange roughy survey than for the oreo survey, which may have increased the total number of species caught on the north of Chatham Rise. Hill (1973) predicated that as the size of a sample is increased, so almost without limit will the diversity. Diversity also declined with increased depth on Chatham Rise, and Crowder (1990) suggested that such a decline in species diversity might be due to changes in the level of competition, predation, or environmental homogeneity.

In this study, only 16 species of shark were caught, although many more species of deep-sea shark have been captured in New Zealand (Paulin et al., 1989). The total of only 16 species caught in the present study is also low in comparison to numbers (>30) of species caught in deep-water surveys in other parts of the world (Kobayashi, 1986; Compagno et al., 1991; Nakaya and Shirai, 1992; Yano and Kugai, 1993). Much of the fishing on Chatham Rise was conducted at depths of greater than 1000 m, which may be beyond the depth limit of a number of species of squalid sharks found in New Zealand waters (Yano, 1985; Compagno et al., 1991).

The index of similarity between the orange roughy and oreo surveys was high, again suggesting that differences introduced as a result of variable fishing methods or time were probably not substantial. The nearly identical indices of similarity between depth intervals for each survey also support this conclusion. The high indices of similarity between areas for most species, along with the fairly low indices of dispersion, indicate that although their abundance is variable, most species have fairly wide distributions on Chatham Rise.

Sharks within the genera *Etmopterus* and *Centrophorus* are thought to segregate by species in Japanese waters (Kobayashi, 1986; Baba et al., 1987; Yano and Tanaka, 1983). In the present study there was little evidence to suggest that any two species displayed such segregation. Compagno et al. (1991) found that *Centroscymnus* spp. were sympatric but had very different food habits. An examination of dietary overlap among sharks common on Chatham Rise may reveal whether these sympatric species compete for the same food resources.

Although the sharks captured during this study were incidental to commercially important fishes, such as orange roughy and smooth oreo, the data collected from these trawls have provided information on the abundance and distribution of a number of species of deep-sea shark on Chatham Rise. Distributional patterns of sharks vary among species, and the composition of the deep-sea shark community varies with depth and location. Therefore, the overall impact of deep-water trawl fisheries on shark populations would be expected to vary among species and to depend on the particular fishery, which in turn influences the location and depth where fishing is concentrated.

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Growth, population age structure, and aspects of the reproductive biology of snowy grouper, *Epinephelus niveatus*, off North Carolina and South Carolina*

David M. Wyanski
D. Byron White
Charles A. Barans
South Carolina Department of Natural Resources
Marine Resources Research Institute
P.O. Box 12559
Charleston, South Carolina 29422-2559
E-mail address (for D. M. Wyanski): wyanskid@mrd.dnr.state.sc.us

Abstract.–Sagittae (n=2,263) and gonads (n=870) from snowy grouper, *Epinephelus niveatus*, caught primarily with longlines, Kali poles, snapper reels, and chevron traps off North Carolina and South Carolina were examined 1) to compare growth rates, population age structure, and sex ratio between two periods 1979–85 and 1993–94, and 2) to determine reproductive seasonality, size and age at maturity, and size and age at sex change. There were several indications that the population off North Carolina and South Carolina is overfished: 1) size at age of specimens caught with longlines and snapper reels has increased noticeably since the 1980s (possibly a a density-dependent population response to a high level of fishing mortality); 2) 81% of the fish caught with commercial longlines during 1993–94 were ages 1–6, the majority (56%) of which were immature females; 3) the percentage of males in the population appears to have decreased significantly, from 7–23% in the 1970s and 1980s to 1% in the 1990s; and 4) mean length of fish landed in the longline fishery has steadily decreased from 65–80 cm in the early 1980s to 50–60 cm in the mid-1990s. There was a positive trend between water depth and total length in fishery-independent samples. Histological examination of gonads revealed that mature gonads were present in 4% of the females at age 3, 52% at age 5, 95% at age 7, and 100% at ages >7 during 1993–94. The smallest mature female was 469 mm TL, and the largest immature female was 575 mm. Estimates of $Length_{50}$ and Age_{50} were 541 mm (95% CI=529–553 mm) and 4.92 yr (95% CI=4.65–5.21 yr), respectively. Spawning females were caught during April through September on the upper continental slope off South Carolina at depths of 176–232 m. The size (767–1090 mm) and age (8–29 yr) of 97 male specimens and the capture of two specimens undergoing sex change provided conclusive evidence that snowy grouper are protogynous hermaphrodites.

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The snowy grouper, *Epinephelus niveatus*, is a commercially important deepwater species that occurs in the western Atlantic from North Carolina (Cape Hatteras) to Brazil, including the Gulf of Mexico and the Bahamas (Smith, 1971). It also occurs in the eastern Pacific from California to Mexico (Miller and Lea, 1976; Fitch and Schultz, 1978). Along the coast of the southeast United States, adult snowy grouper are predominantly found on the upper continental slope (>75 m; Lee et al., 1985) at depths of 116–259 m (Low and Ulrich, 1983; Moore and Labisky, 1984; Parker and Ross, 1986), whereas juveniles are more common at shallower depths (Moore and Labisky, 1984). Low and Ulrich (1983) noted a positive correlation between total length (TL) and water depth off South Carolina. Most fishing for this species occurs in habitats characterized by rocky ledges, cliffs, and swift currents (Matheson and Huntsman, 1984).

Snowy grouper are captured primarily in commercial fisheries of the southeastern United States (Parker and Mays, 1998). Most are caught with bottom longlines and snapper reels[1] (handlines). Starting in 1991, the longline fishery was restricted to waters deeper than 91 m by the South Atlantic Fishery Management Council (SAFMC, 1991). Fishing for snowy grouper has occurred off North Carolina and South Carolina (the Carolinas) since the mid-1950s (Huntsman, 1976); annual landings from the Carolinas averaged 119,657 kg during 1981–96 (Moran[2]). According to the spawning stock ratio (SSR), the population off the Atlantic coast of the United States is considered to be overfished (SAFMC, 1993).

Population age structure and individual growth rates in the snowy grouper population have not been assessed since the mid-1980s and should be assessed again given the sustained fishing pressure on the population. Studies of other fish populations have shown that size at age is often affected by the level and duration of exploitation (Haug and Tjemsland, 1986; Harris and McGovern, 1997; Helser and

* Contribution 430 of the South Carolina Marine Resources Center, P.O. Box 12559, Charleston, SC 29422.

[1] Snapper reels are commonly known as "bandits" or "one-armed bandits" by fishermen owing to the remote similarity between early snapper reels and gambling slot machines, and because luck is involved in what is caught. The early mechanical reels have typically been replaced by 12 volt DC automobile starter motors or hydraulic systems.

[2] Moran, J. 1996. S. Carolina Dept. of Natural Resources, P.O. Box 12559, Charleston, SC 29422. Personal commun.

Almeida, 1997; Zhao et al., 1997; Goodyear and Schirripa[3]), thus trends in growth can be indicators of population stability. Additionally, nothing is known about trends in sex ratio. Recent studies along the southeast Atlantic coast and in the Gulf of Mexico have documented sharp decreases in the proportion of males in other grouper, gag (*Mycteroperca microlepis*) and scamp (*M. phenax*), populations (Coleman et al., 1996; McGovern et al., 1998). This and other factors have led to reduced genetic diversity in the gag population along the southeast coast, a cause for serious concern, although the ramifications are not clearly understood (Chapman et al., 1999).

Little is known about the reproductive biology of snowy grouper off the Carolinas because the only previous study (Moore and Labisky, 1984) was conducted in the Florida Keys. Using a histological technique, Moore and Labisky (1984) showed that the snowy grouper is a protogynous hermaphrodite; females reach sexual maturity at ages 3–5 and change to males as early as age 6. The objectives of the present study were 1) to compare individual growth rates, population age structure, and sex ratio between two periods, 1979–85 and 1993–94, and 2) to determine reproductive seasonality, size and age at maturity, and size and age at sex change for snowy grouper off the Carolinas.

Materials and methods

Specimen acquisition

Snowy grouper were obtained from commercial boats, research vessels, and headboats, primarily off North Carolina and South Carolina (Table 1). All specimens were collected between 31°09'N and 34°44'N at depths of 42–302 m. Only seven specimens were caught south of 32°04'N. Fishery-independent samples were collected during cruises of the MARMAP (Marine Resources Monitoring Assessment and Prediction) program with bottom longlines, Kali poles (an off-bottom longline; Russell et al., 1988), snapper reels, rods and reels, and chevron traps (Collins, 1990). Specimens caught with longlines were collected primarily off South Carolina, whereas specimens caught with snapper reels were collected off South Carolina in the 1980s and primarily off North Carolina in the 1990s (Fig. 1). Total length (mm) was measured for all specimens and all length measurements in the text refer to total length (TL).

During 1993–94, samples from the commercial fishery usually included the total catch of snowy grouper from a vessel or a random subsample; on three of 20 occasions, the catches from vessels that landed fish in South Carolina were subsampled to collect otoliths from small and large specimens. No documentation on sampling design was available for fishery-dependent samples that were collected with snapper reels during 1979–85.

Age and growth

The left sagitta, and the right sagitta when time permitted, was removed and stored dry prior to processing. Each otolith was embedded in paraffin (1979–85) or Araldite epoxy resin (1993–94) and sectioned along a dorsoventral plane through the focus with a single high-concentration diamond wheel on a Buehler Isomet low-speed saw. Otolith sections were mounted on glass slides with Crystalbond thermoplastic or Accu-mount 60, covered with cedar wood oil, and examined under a dissecting microscope (10–63×) with reflected and transmitted light.

We examined otoliths from 1937 specimens and used the age determinations (n=326) of Waltz[4] for specimens collected with snapper reels from 1979 to 1985. The width of the translucent zone along the margin of the section was measured to assess the periodicity of increment formation. Increments were counted independently by two readers for 1853 of 1937 specimens. In older fish, all increments could not be counted along one axis in many specimens. Counting commenced on one of three axes (ventral, ventromedial, or adjacent to the sulcus acousticus) and shifted to another axis by following an increment to the new axis. If counts differed, both readers examined the otolith by projecting the image onto a TV monitor. The otolith was rejected if agreement could not be reached.

A small portion (n=129) of the 1937 otoliths that we examined were used for an earlier MARMAP study (Waltz[4]). Age assessments were compared to determine if annual increment structure was being interpreted in a similar manner. The specimens selected for the comparison were collected with longlines or Kali poles on research cruises during 1982–85. Age data from specimens collected with these two gear types were combined because they were fished simultaneously in the same area and were deployed with the same hook type and bait.

The sagittae of three young-of-the-year (YOY) specimens were hand-polished to thin (approx. 5μm)

[3] Goodyear, C. P., and M. J. Schirripa. 1993. The red grouper fishery of the Gulf of Mexico, Report MIA 92/93-75. Miami Laboratory, Southeast Fisheries Science Center, National Marine Fisheries Service, 75 Virginia Beach Dr., Miami, FL 33149.

[4] Waltz, W. 1986. The size and age of snowy grouper (*Epinephelus niveatus*) in the South Atlantic Bight. MARMAP Analytical Report, 16 p. S. Carolina Department of Natural Resources, P.O. Box 12559, Charleston, SC 29422.

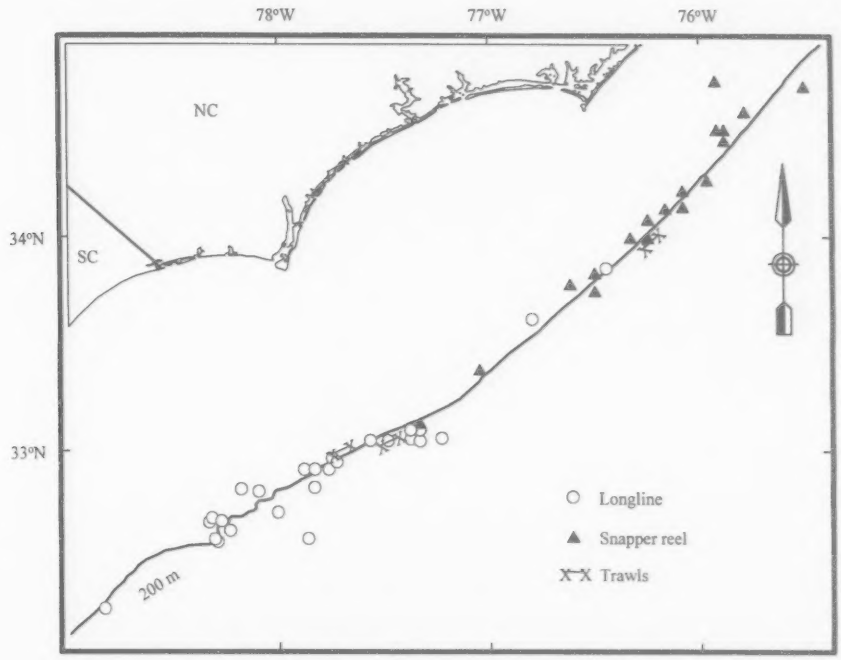


Figure 1

Approximate locations of commercial fishing effort with longlines and snapper reels for snowy grouper off North Carolina and South Carolina between June 1993 and September 1994. Snowy grouper were also caught in trawls in June 1978 during an exploratory squid cruise conducted by the government of Spain and the National Marine Fisheries Service on the RV *Pescapuerta Segundo*. Trawl site off Georgia is not shown.

Table 1

Numbers of specimens of snowy grouper for which otoliths and gonad samples were examined by gear type. C = commercial landings; H = headboats sampled by National Marine Fisheries Service, Beaufort, North Carolina; R = research cruises conducted by Marine Resources Monitoring Assessment and Prediction Program (MARMAP).

Gear	Source	Period	Otoliths	Gonads
Snapper reel	C, R	1979–85	326	309
Longline and Kali pole	most R, C	1982–85	190	180
Rod and reel	most H, R	1973–81	0	90
Other	C, R	1980–84	0	10
Snapper reel	C	1991–95	335	32
Longline	C	1993–94	1332	146
Chevron trap	R	1991–95	78	100
Other	R	1993–95	2	3
Total			2263	870

transverse sections according to the methods of Secor et al. (1992) and examined with a compound microscope. We counted the regular concentric rings, similar to those reported as daily growth increments in other species, to estimate age. Assumed daily rings were visible, with the exception of a large opaque core area, on the smallest otolith but were only distinguishable at discrete locations on the other two otoliths. For these two otoliths, age was estimated by extrapolating the increment count per unit distance to the total radial measurement of the ventral axis, excluding the core. Radial measurement

from the core to the first annulus was made for a subsample of 23 specimens that were age 1 and compared with the measurements from the three YOY to establish the position of the first annulus.

Age-length keys were formed by obtaining a matrix of numbers at age by length interval for each gear type (longline and snapper reel) in two periods (1980s and 1990s). Besides the differing selectivities of longlines and snapper reels, another reason for developing keys by gear type was the difference in sampling area in 1993–94 (Fig. 1) and potential differences in sampling depth due to the restriction (SAFMC, 1991) that limits the use of longlines to waters deeper than 91 m. Additional keys were generated to address two questions: 1) Do data from specimens with age estimates of lower precision affect the accuracy of the key for specimens caught with longlines in the 1990s? and 2) Are there differences in the keys for specimens caught with longlines or Kali poles during 1982–85 that were examined in the present study and in an earlier study by Waltz?[4] To address the first question, keys based on all specimens and specimens for which there was a difference of 0–1 increments between readers were compared.

All analysis of age and growth data was conducted with SAS software (SAS Institute, Inc., 1990). Fisher's exact test (Siegel, 1956) was used to compare the age distributions of two age-length keys by 25-mm length intervals. The FREQ procedure was used to run this test. A comparison was made only if each key had >6 specimens in an interval because of the low power associated with small sample sizes (Bennett and Hsu, 1960). The large number of tests required to compare age-length keys necessitated compensating for experimentwise error by computing an adjusted significance level (α^*) using the formula presented by Hayes (1993).

Nonlinear regression analysis with Marquardt's algorithm (NLIN procedure) and the NLIN weight statement were used to fit the von Bertalanffy growth model to observed length at age data (von Bertalanffy, 1938). Lengths were weighted by the inverse of the number of fish at each age to moderate the effect of large and small sample sizes on the estimates of growth parameters. Age-length keys were applied to length data collected through the Trip Interview Program (TIP) in the Carolinas to generate an age-frequency distribution. TIP is a commercial fisheries data collection program funded by the National Marine Fisheries Service (NMFS).

Reproduction

Gonads were obtained during 1979–95 from 870 specimens collected on research cruises and from fish landed whole by fishermen (Table 1). Ninety gonad samples from the headboat fishery during 1973–1981, collected in association with the study of Matheson and Huntsman (1984), were obtained from the Beaufort Laboratory of the NMFS. The posterior portion of the gonad was fixed in 10% seawater-formalin for 1–2 weeks and transferred to 50% isopropanol for 1–2 weeks. Gonad samples were processed, sectioned, and stained with double-strength Gill hematoxylin and eosin-y by using the methods of Schmidt et al. (1993).

Sex and reproductive state were assessed primarily by one reader using histological criteria (Table 2), without reference to body length or date of capture. A second reader examined sections from 75 specimens to ensure accurate interpretations. If the assessments of the two readers differed, the section was viewed simultaneously by the readers and rejected if agreement could not be reached. Specimens with developing, ripe, spent, or resting gonads were considered sexually mature. For females, this definition of sexual maturity included specimens with oocyte development at or beyond the cortical granule stage and specimens with beta, gamma, or delta stages of atresia (see Hunter and Macewicz, 1985). To ensure that females were correctly assigned to either the immature or resting categories, the length-frequency histogram of females with evidence of certain maturity (e.g. those that were developing, ripe, or spent) was compared with the histograms for immature and resting females. Females of uncertain maturity (Table 2) were excluded from data analyses. To estimate length at 50% maturity (L_{50}) and age at 50% maturity (A_{50}), the PROBIT procedure (SAS Institute, Inc., 1990) was used to fit gompit, logit, or probit models to maturity data in 25-mm length intervals or one year increments. The LOGISTIC procedure was used to determine which model to use in the PROBIT procedure.

Females with hydrated oocytes or postovulatory follicles were considered to be in spawning condition. Macroscopic observations of snowy grouper caught in June 1978 during trawls (Fig. 1) of an exploratory squid cruise conducted jointly by the government of Spain and the Northeast Fisheries Center of NMFS in Woods Hole, Massachusetts, on the RV *Pescapuerta Segundo* were also used to define the area and timing of spawning.

Results

Age and growth

An age was assigned to 91.6% of 1937 otoliths that we examined (Table 1). Otoliths were rejected if the

Table 2

Histological criteria used to determine reproductive stage in snowy grouper (see Hunter and Goldberg, 1980; Wallace and Selman, 1981; Hunter and Macewicz, 1985; Wenner et al., 1986; West, 1990).

Reproductive stage	Criteria
Immature	Previtellogenic oocytes only, no evidence of atresia. In comparison with resting female, most previtellogenic oocytes are <70 µm, area of transverse section of ovary is smaller, lamellae lack muscle and connective tissue bundles and are not as elongate, oogonia are abundant along margin of lamellae, and ovarian wall is thinner.
Developing	Oocytes undergoing cortical granule (alveoli) formation through nucleus migration and partial coalescence of yolk globules.
Ripe	Completion of yolk coalescence and hydration in the most advanced oocytes. Zona radiata becomes thinner.
Developing, recent spawning	Developing stage as described above plus presence of postovulatory follicles.
Spent	More than 50% of vitellogenic oocytes in alpha or beta stage of atresia.
Resting	Previtellogenic oocytes only with traces of atresia possible. In comparison with immature female, most previtellogenic oocytes >70 µm, area of transverse section of ovary is larger, lamellae have muscle and connective tissue bundles, lamellae are more elongate and convoluted, oogonia are less abundant along margin of lamellae, and ovarian wall is thicker and exhibits varying degrees of expansion owing to previous spawning.
Uncertain maturity	Immature or resting. Inactive ovaries, previtellogenic oocytes only. Reproductive stage is uncertain.
Transitional	Proliferation of spermatogonia through limited spermatogenesis within lamellae of resting ovary, accompanied by development of peripheral sinuses in musculature of ovarian wall.

readers could not agree on the age or if the section was not adequate. Ages ranged from 1 to 29 yr and lengths from 226 to 1137 mm. From a subsample (n=274) of 3–10 yr old specimens, we found that the mean width of the sagittal marginal translucent zone was smallest in April and May, indicating the period of increment formation (Fig. 2). The unimodal nature of the data indicated that one increment was deposited per year.

Data from longlines and snapper reels showed that size at age was greater during 1993–94 than during the previous decade (Figs. 3 and 4). Snowy grouper captured with longlines in 1993–94 exhibited a nearly constant growth rate until approximately age 10, after which there was a notable decrease. A similar growth pattern was noted for snowy grouper caught with snapper reels, although the trend was less definitive owing to smaller sample sizes. Estimates of theoretical maximum length (L_∞) were reasonable when compared with maximum observed lengths (Table 3). Data from both gear types indicated that L_∞ has increased 169–231 mm in the last decade. The application of the age-length key for samples caught with longlines during 1993–94 to TIP length data for the same period revealed recruitment to the fishery as early as age 1, and a modal age for recruitment of 5 (Table 4).

The increase in size at age since the 1980s was also evident in comparisons of age-length keys between

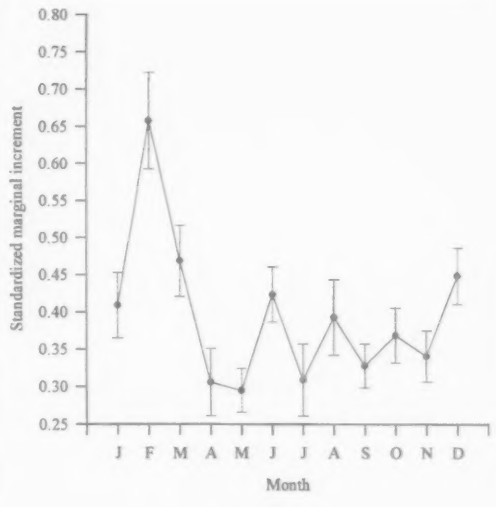


Figure 2

Mean width (±SE) of marginal translucent zone in transverse sections of sagittae from 248 snowy grouper that were 3–10 yr old.

periods for each gear type. For longline gear, the comparisons in 8 of 8 length intervals exceeded the adjusted significance level (P<0.00639; Table 5). The

Table 3

Von Bertalanffy parameters (± SE) describing the growth in mm total length of snowy grouper collected with snapper reels and longlines and Kali poles (LL, KP) during two decades. Mean observed length at age data were used to generate parameter estimates. Estimates from two earlier studies, generated using back-calculated lengths, are included for comparison.

Study	Gear and source	Period	n	Maximum observed length	L_∞	k	t_0
Waltz (1986)	Snapper reels commercial, research	1979–85	326	1090	970 (24)	0.109 (0.001)	–2.123 (0.336)
Present	Snapper reels commercial	1993–94	311	1110	1201 (34)	0.103 (0.008)	–1.149 (0.231)
Present	LL, KP, research	1982–85	163	1034	948 (28)	0.122 (0.017)	–0.668 (0.681)
Present	LL, commercial	1993–94	1218	1137	1117 (13)	0.119 (0.004)	–1.409 (0.121)
Matheson and Huntsman (1984)	Hook and line, headboat	1972–79	536	1130	1255	0.074	–1.92
Moore and Labisky (1984)	Hook and line, research	1978–81	118	1180	1320	0.087	–1.013

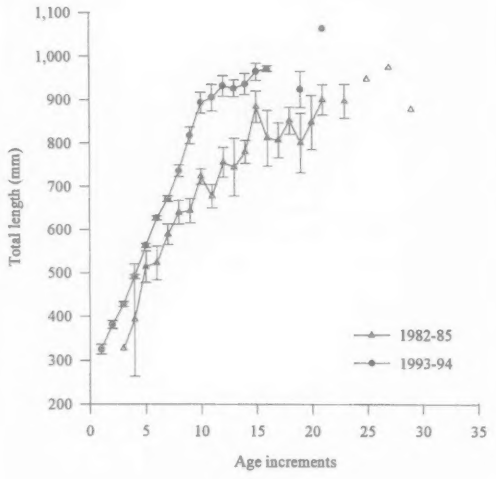


Figure 3

Mean observed size at age (±SE) for snowy grouper collected with longlines and Kali poles during 1982–85 (n=163) and longlines during 1993–94 (n=1,218) off North Carolina and South Carolina.

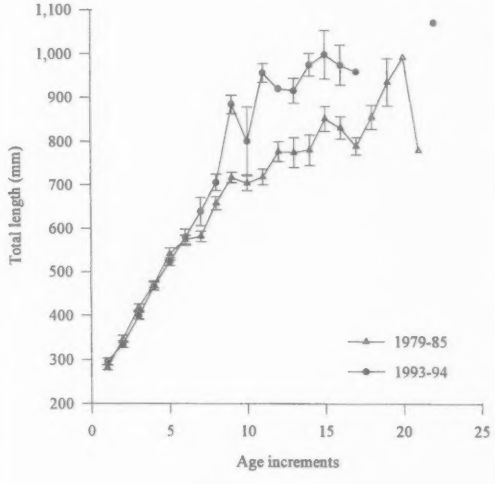


Figure 4

Mean observed size at age (±SE) for snowy grouper collected with snapper reels during 1979–85 (n=326) and 1993–94 (n=311) off North Carolina and South Carolina.

trend was not as strong for snapper reel gear because comparisons in 5 of 13 intervals exceeded the 0.05 level; only one comparison exceeded the adjusted significance level (P<0.00394; Table 5). The age-length keys for the two gear types were very similar in 1993–94 because comparisons in only 3 of 17 intervals exceeded the 0.05 level, none of which exceeded the adjusted significance level (P<0.00284; Table 5).

Initial agreement between independent readers was 24.2% for the 1853 otoliths examined by two readers; however, there was a difference of 0–1 increments between readers for 61.3% of the otoliths. The difference was >1 increment for 30.8% of the otoliths and 7.9% were rejected as uninterpretable. The inclusion in the data sets of age data for otoliths that were difficult to interpret did not affect the assessment of population age distribution for snowy grou-

Table 4

Population age structure of snowy grouper captured with longlines in North Carolina and South Carolina from July 1993 through May 1994. Age-length key was applied to length data from the same period collected during Trip Interview Programs.

Age	Number
1	5
2	32
3	77
4	172
5	209
6	163
7	82
8	23
9	13
10	8
11	6
12	7
13	4
14	2
15	3
16+	3
Total	809

per caught with longlines in 1993–94. Comparisons of age-length keys based on all specimens and specimens for which there was a difference of 0–1 increments between readers revealed a strong similarity in age distribution for 20 intervals ($P>0.00256$; Table 6).

Slightly less initial agreement was noted when our age estimates were compared with those of Waltz[4] for specimens caught in 1982–85 with longlines and Kali poles. Ages were assigned to 85.3% of 129 specimens that were examined in both studies. The same age was assigned to 18.2% of the specimens and there was a difference of 0–1 increments between studies for 40.0% of the specimens. Although the percent agreement was low, the estimates of mean size at age were similar (Fig. 5). Age distributions in the five length intervals that could be tested were similar ($P>0.01021$; Table 6). Given the similarity of age distributions, we decided to use the age data of Waltz[4] for snowy grouper caught with snapper reels in 1979–85 without reexamining the otoliths.

The low initial agreement between readers was due to a lack of a readily discernible growth pattern in many otoliths. Typical abnormalities included crystalline areas that obscured increments (Fig. 6A) and rounded opaque deformities that distorted increment spacing (Fig. 6B). In addition, the axis of otolith growth frequently changed direction at least once after 6–7 increments (Fig. 6C). Despite our inability to make linear measurements for back calculations, counts of increments were usually possible, although easily interpreted otoliths (Fig. 6D) were the exception.

We attempted to identify the first annulus 1) by examining daily increment structure in the sagittae of three specimens that were most likely YOY, and

Table 5

Comparison of age distributions by length interval with Fisher's exact test for snowy grouper collected off North Carolina and South Carolina with snapper reels during two periods (1979–85 and 1993–1994), with longlines during two periods (1982–85 and 1993–94), and with longlines and snapper reels during 1993–94. Dashed lines indicate that the sample size was less than seven in one or both groups.

mm TL	Snapper reel, 1979–85 vs. 1993–94	Longline, 1982–85 vs.1993–94	Longline vs. snapper reel, 1993–94
226–250	—	—	—
251–275	—	—	—
276–300	—	—	0.177
301–325	0.570	—	0.841
326–350	—	—	0.074
351–375	0.020	—	0.488
376–400	0.933	—	0.730
401–425	0.057	—	0.407
426–450	1.000	—	0.502
451–475	0.021	—	0.036
476–500	0.630	—	0.595
501–525	0.430	—	0.043
526–550	0.019	—	0.753
551–575	0.001*	—	0.323
576–600	0.209	—	0.972
601–625	0.045	<0.001*	0.200
626–650	—	<0.001*	0.668
651–675	0.064	<0.001*	0.438
676–700	—	<0.001*	—
701–725	—	<0.001*	0.005
726–750	—	0.002*	—
751–775	—	<0.001*	—
776–800	—	—	—
801–825	—	0.003*	—
826–850	—	—	—
851–875	—	—	—
876–900	—	—	—
901–925	—	—	—
926–950	—	—	—
951–975	—	—	—
976–1000	—	—	—
α^1	0.00394	0.00639	0.00284

* $P < \alpha^1$. α^1 = adjusted significance level.

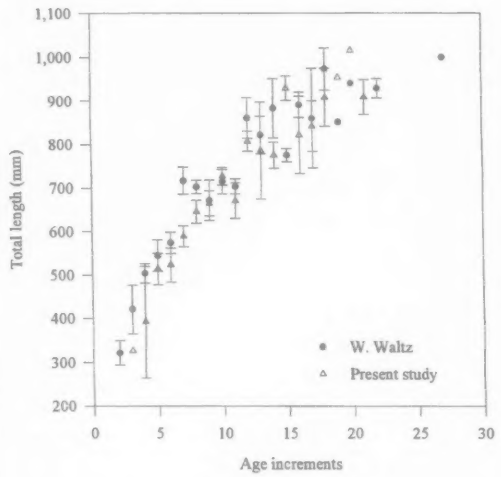


Figure 5

Mean observed size at age (±SE) for snowy grouper caught with longlines and Kali poles in the 1980s. The sagittae were examined by Waltz (n=132; see Footnote 3 in the main text) and investigators in the present study (n=129).

2) by comparing the measurements of otolith radius in these three specimens with the radial measurement to the first annulus in a subsample of 23 specimens that were age 1. Examination of sagittae from YOY revealed that fish lengths of 37, 156, and 172 mm were associated with estimated ages of 35, 159–201 and 191–291 days, respectively. Radial measurement to the edge in these three otoliths was less than the radial measurement to the first annulus in a subsample of 23 age-1 specimens.

Reproduction

Histological examination of 599 sexually mature snowy grouper in four data sets from three periods (1970s, 1980s, and 1990s) suggested that the number of males in the population has substantially decreased. The percentage of males was 7.2%, 19.5%, and 22.9% for samples collected with hook and line during 1973–81, snapper reels during 1980–84, and longlines and Kali poles during 1982–85, respectively, whereas males represented 1.2% of samples collected with longlines during 1993–94 (Table 7). Although sample sizes were <100 for two data sets, especially the 1993–94 data set, the mean total length ($\bar{x}$=63.6 cm, SE=1.0) of specimens in the 1993–94 data set was larger than the mean length of specimens measured through the TIP in North Carolina (1993: $\bar{x}$=58.0 cm, SE=0.6; 1994: $\bar{x}$=56.0, SE=1.4) and South Carolina (1993: $\bar{x}$=59.1, SE=0.7; 1994: $\bar{x}$=53.8, SE=0.6) for the same years, indicating that our data set was not biased toward smaller specimens.

There were ninety-seven males and two transitional specimens in the four sex-ratio data sets.

Table 6

Comparison of age distributions by length interval with Fisher's exact test for snowy grouper collected off North Carolina and South Carolina. These comparisons included 1) all specimens that were caught with longlines during 1993–94 and assigned an age versus those for which the difference in age assignment between readers was 0–1 increments (Best), and 2) specimens caught with longlines during 1982–85 that were examined in the present study and by Waltz (Footnote 4 in the main text). Dashed lines indicate that the sample size was less than seven in one or both groups.

mm TL	Best vs. all	Longline, present study vs. Waltz
251–275	—	—
276–300	—	—
301–325	—	—
326–350	1.000	—
351–375	1.000	—
376–400	0.852	—
401–425	0.855	—
426–450	0.980	—
451–475	0.978	—
476–500	0.951	—
501–525	0.853	—
526–550	0.966	—
551–575	0.932	—
576–600	0.997	—
601–625	0.993	0.669
626–650	1.000	0.669
651–675	0.955	—
676–700	0.663	—
701–725	0.832	0.242
726–750	0.943	1.000
751–775	0.902	0.660
776–800	—	—
801–825	1.000	—
826–850	0.795	—
851–875	—	—
876–900	—	—
901–925	—	—
926–950	—	—
951–975	—	—
976–1000		—
α^1	0.00256	0.01021

α^1 = adjusted significance level.

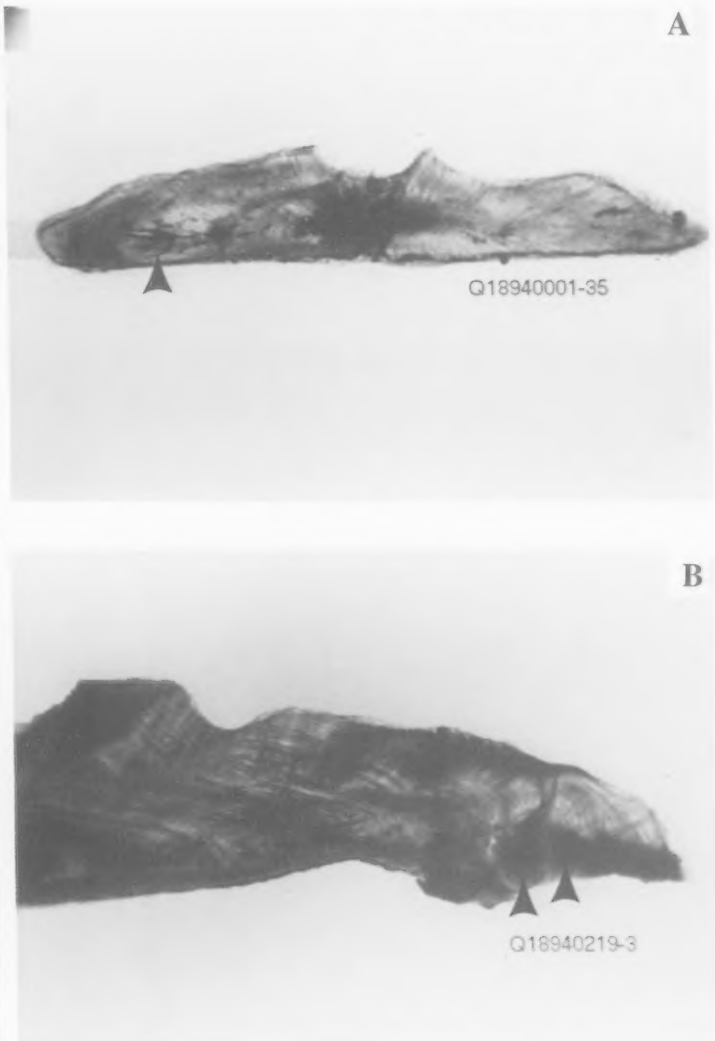
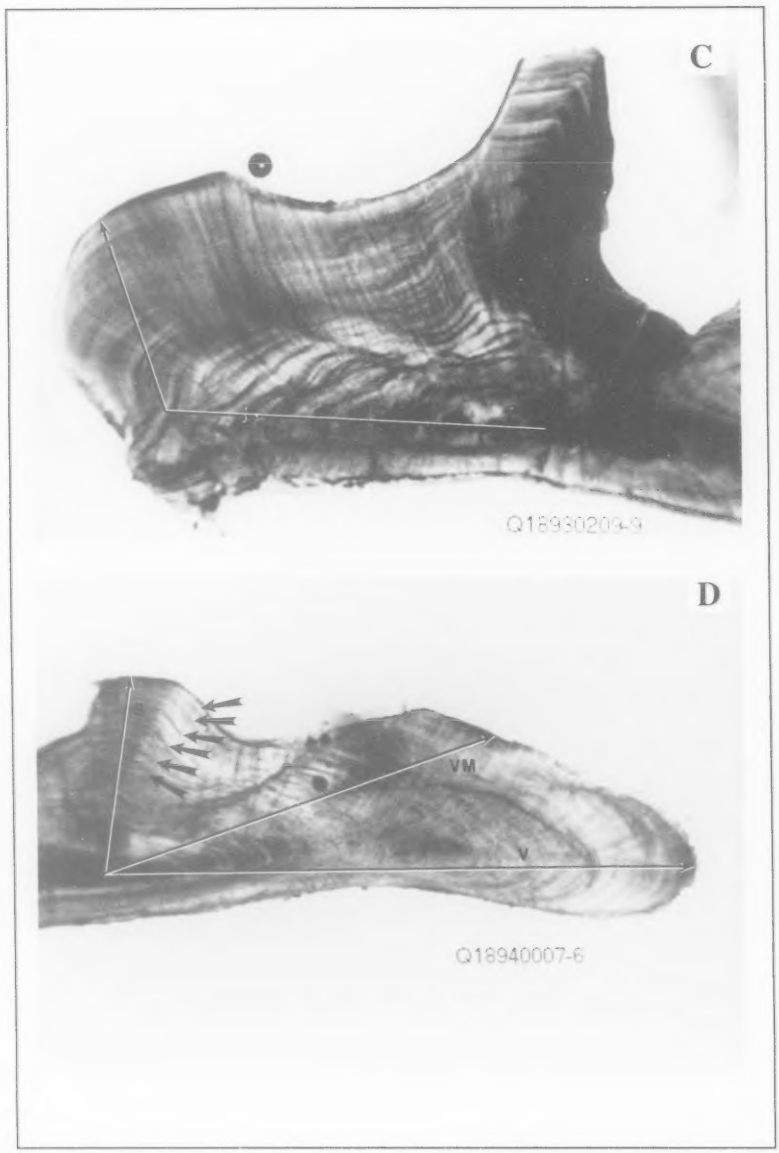


Figure 6

Transverse sections of snowy grouper sagittae displaying (**A**) crystalline area in an age-1 fish that obscures increments, (**B**) opaque deformities (arrows) in an age-5 fish that distort increment spacing, (**C**) change in growth axis in an age-9 fish, and (**D**) easily interpreted increments in an age-6 fish. S = sulcus acousticus axis, VM = ventromedial axis, V = ventral axis.

Males were collected during March through September and their length range was 767–1090 mm (median=918, $\bar{x}$=919 mm, SE=7). Age was assessed for 47 males and ranged from 8 to 29 yr (median=16, $\bar{x}$=16.87, SE=0.63). Transitional specimens (787 mm and unknown age, 1000 mm and age 13) were collected in July and September. The 787 mm specimen was in the latter stage of sexual change (Fig. 7); male tissue was predominant, although previtellogenic oocytes were still numerous.

The near overlap in the length distributions of female snowy grouper that were definitely mature and females that were resting indicated that specimens were correctly assigned to immature and rest-



ing categories (Fig. 8); only 29 females of uncertain maturity were excluded from analyses. Samples collected primarily with longlines and chevron traps in 1991–95 revealed that snowy grouper become sexually mature at lengths of 451 to 575 mm (ages 3–7; Tables 8 and 9). Mature gonads were present in 4% of females at age 3, 52% at age 5, 95% at age 7, and 100% at ages >7 (Table 9). The smallest mature female was 469 mm, and the largest immature female was 575 mm. Estimates of L_{50} and A_{50} were 541 mm (gompit model; 95% CI=529–553 mm) and 4.92 yr (probit model; 95% CI=4.65–5.21 yr).

Samples collected primarily with bandit reels and longlines in 1980–85 showed that snowy grouper reached sexual maturity at similar lower limits of length and age, 476–500 mm and age 3, although sample sizes were small (<10) in the length and age intervals near the lower limit (Tables 8 and 9). Upper limits of size and age at maturity were higher, 626–650 mm and age 9, during this period and better defined owing to larger sample sizes in comparison to those for 1991–95. Mature gonads were present in 33% of females at age 3, 62% at age 5, 91% at age 7, 95% at age 9, and 100% at ages >9 (Table 9). The smallest

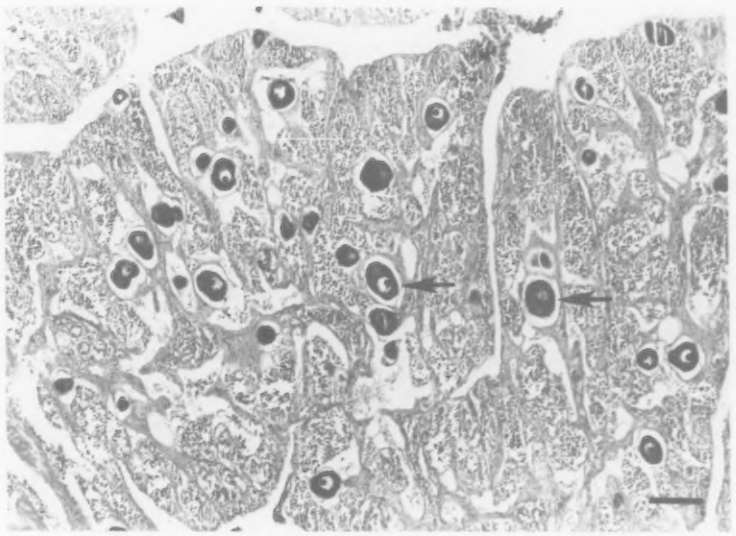


Figure 7

Histological section of gonad tissue from a 787-mm-TL snowy grouper captured in July in which transition to male is nearly completed. Chromatin nucleolar (arrows) and perinucleolar oocytes are still present. Bar = 100µ.

Table 7

Sex ratios of snowy grouper, *Epinephelus niveatus*, (M= male; F=female; T=transitional) collected off North Carolina and South Carolina during 1973–94. Ratios based on sexually mature individuals. LL=longline; KP=Kali pole.

Gear and source	Period	*n*	%*M*	%*F*	%*T*
Rod and reel headboat	1973–81	83	7.2	91.6	1.2
Snapper reels commercial, research	1980–84	281	19.5	80.5	0.0
LL, KP, research	1982–85	153	22.9	76.4	0.7
LL, commercial	1993–94	82	1.2	98.8	0.0

mature female was 483 mm, and the largest immature female was 634 mm. The estimate of L_{50} was 486 mm (logit model; 95% CI=449–509 mm) and A_{50} was not estimated owing to the absence of specimens younger than age 3. A third data set, specimens collected by NMFS during 1973–81 primarily from headboats, exhibited a pattern of size at maturity similar to that found for the 1980–85 samples, though sample sizes were <10 in every length interval and only three specimens were <551–575 mm (Table 8).

Snowy grouper were in spawning condition from April through September based on the presence of hydrated oocytes (Fig. 9A) and postovulatory follicles (Fig. 9B), with no obvious peak period (Fig. 10). Given the small sample sizes for October through March, the spawning season could be longer. Ninety-nine female specimens were captured in spawning condition. Seventy-two percent of the specimens were collected on research vessels off South Carolina (32°28' to 32°50'N) at depths of 176–232 m, primarily during May and July through September. The remaining 27 fish in spawning condition were collected during April through August on headboats off South Carolina, on research vessels off North Carolina between Cape Fear and Cape Lookout, and by commercial fishermen on the upper continental slope off North and South Carolina; exact location data were not recorded. Commercial fishermen reported approximate locations of 32°36' to 33°51'N and depths of 189–302 m for spawning fish.

Trawl collections during exploratory squid cruises in June 1978 also provided evidence that snowy grouper spawn on the upper continental slope (Fig. 1). Four large catches (1160 fish/8776 kg), which were made at depths of 180–316 m off North Carolina, South Carolina, and Georgia (not shown), ranged from 90 to 520 snowy grouper per tow. Tow distance ranged from 7.4 to 18.5 km and estimates of snowy grouper density ranged from 2.2 fish/ha to 10.9 fish/ha (13.5 kg/ha to 79.5 kg/ha). Although the reproduc-

Table 8

Percentage of mature specimens by length interval for female snowy grouper collected off North Carolina and South Carolina with 1) primarily bandit reels and longlines during 1980–85, 2) primarily longlines and chevron traps during 1991–95, and 3) primarily from headboats during 1973–81. Specimens in the developing, ripe, spent, or resting stages were considered mature. All specimens were examined histologically. n = number of specimens.

mm TL	1980–85 n = 372 % (n)	1991–95 n = 235 % (n)	1973–81 n = 74 % (n)
251–275	0 (1)	0 (3)	—
276–300	—	0 (6)	—
301–325	0 (1)	0 (10)	—
326–350	0 (1)	0 (12)	—
351–375	—	0 (10)	0 (1)
376–400	0 (5)	0 (11)	—
401–425	0 (3)	0 (11)	—
426–450	0 (1)	0 (17)	0 (1)
451–475	0 (2)	4 (22)	—
476–500	67 (6)	35 (17)	—
501–525	88 (8)	18 (17)	—
526–550	80 (15)	29 (7)	0 (1)
551–575	100 (20)	70 (20)	100 (7)
576–600	90 (21)	100 (13)	86 (7)
601–625	92 (24)	100 (9)	100 (6)
626–650	97 (31)	100 (12)	100 (8)
651–675	100 (25)	100 (7)	100 (9)
676–700	100 (31)	100 (7)	100 (7)
701–725	100 (39)	100 (7)	100 (3)
726–750	100 (35)	100 (4)	100 (6)
751–775	100 (28)	100 (3)	100 (7)
776–800	100 (15)	100 (1)	100 (3)
801–1025	100 (59)	100 (3)	100 (8)
No length	100 (1)	100 (5)	—

tive stage of individual fish was not determined, it was noted that milt flowed freely from males in a collection of 420 fish from a depth of 278 m off South Carolina (32°57' to 33°02'N). Nearly all of the specimens were sexually mature as the mean lengths of subsamples collected off North Carolina and off South Carolina and Georgia combined were 67.3 (48–96 cm; n=89) and 79.2 (60–97 cm; n=98), respectively.

Depth distribution

There was a moderately positive trend (r^2=0.53) between water depth and total length in fishery-independent samples. Snowy grouper were caught at depths of 46–258 m. Larger adults were caught more frequently in upper continental slope waters >100 m, whereas small adults and juveniles (<600 mm) were caught more frequently at depths <100 m (Fig. 11).

Table 9

Percentage of mature specimens by age class for female snowy grouper collected off North Carolina and South Carolina with 1) primarily bandit reels and longlines during 1980–85, and 2) primarily longlines and chevron traps during 1991–95. Specimens in the developing, ripe, spent, or resting stages were considered mature. All specimens were examined histologically. n = number of specimens.

Age (yr)	1980–85 n = 219 % (n)	1991–95 n = 197 % (n)
1	—	0 (5)
2	—	0 (22)
3	33 (3)	4 (26)
4	80 (5)	23 (43)
5	62 (8)	52 (40)
6	86 (21)	83 (29)
7	91 (32)	95 (21)
8	93 (30)	100 (7)
9	95 (20)	100 (1)
10	10 (26)	100 (1)
11	100 (15)	100 (1)
>11	100 (59)	100 (1)

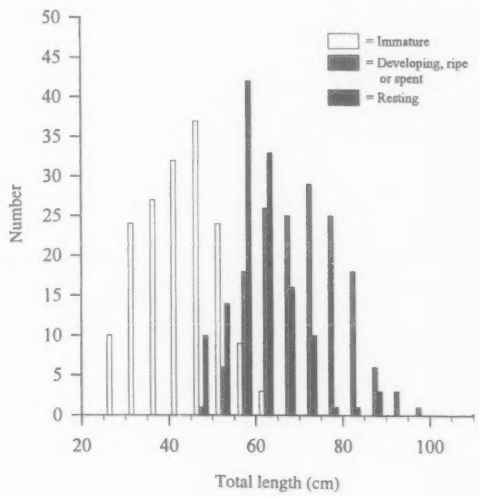


Figure 8

A comparison of length-frequency histograms for snowy grouper specimens collected during 1973–95 that were categorized as immature (n=166), definitely mature (n=158), or resting (n=130). Definitely mature specimens were developing, ripe, or spent.

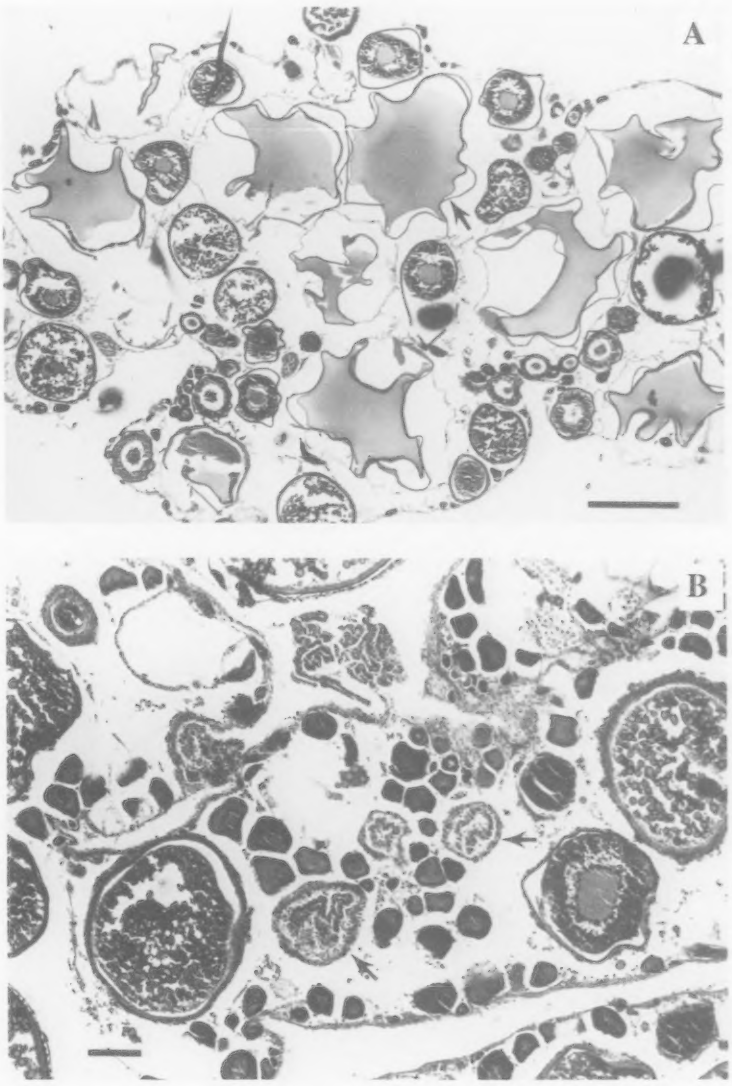


Figure 9

Histological sections of ovarian tissue from female snowy grouper with evidence of imminent or recent spawning: (**A**) hydrated oocytes (arrow) in a 744-mm-TL specimen captured in May (bar=400μ), and (**B**) age 24–48 h postovulatory follicles (arrows) in a 546-mm-TL specimen captured in August (bar=100 μ).

The fishery

Historically, most snowy grouper landings along the Atlantic coast of the United States, as reported through TIP, have occurred in North Carolina and South Carolina (Fig. 12). Although landings statistics are reported by state, fish are often caught throughout the region, especially by vessels fishing with longlines. Landings have varied widely, with peaks noted in 1983 and 1990 for South Carolina and in 1990 and 1992 for North Carolina. These fluctuations have often been the result of changes in effort. For example, the peak in 1983 reflected an approximate doubling in the number of vessels as

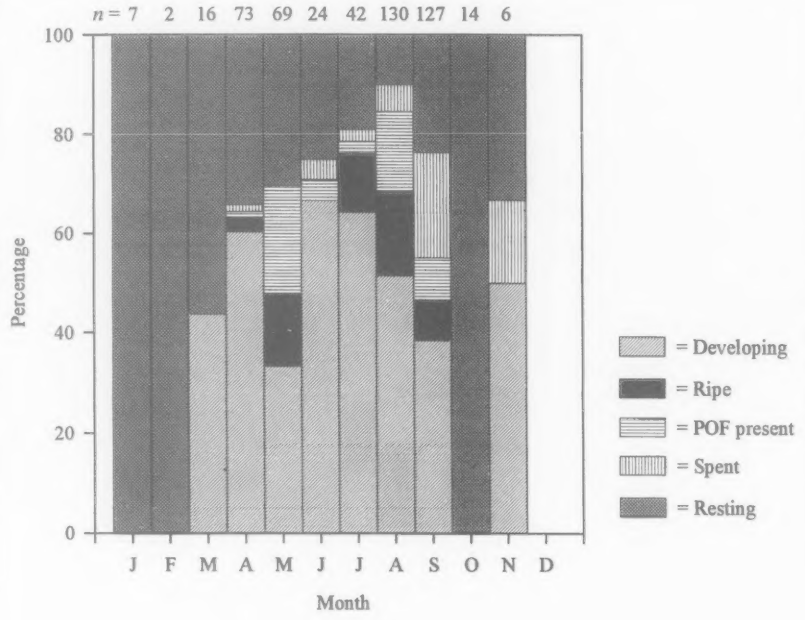


Figure 10

Percentage composition (based on histological criteria) of reproductive stages by month for 510 female snowy grouper. Number of specimens examined is above each bar. POF = postovulatory follicle.

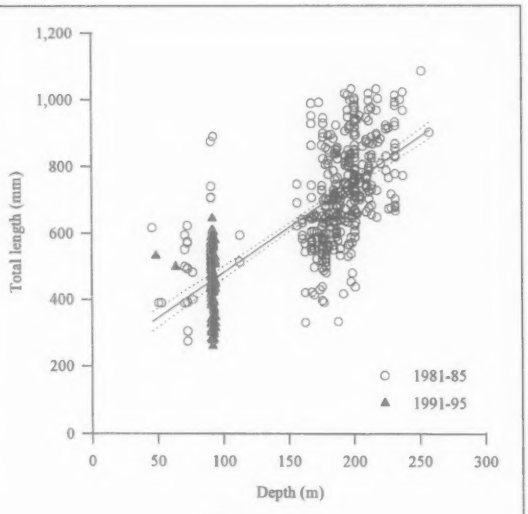


Figure 11

Relationship between length of snowy grouper and depth of capture in fishery-independent sampling off North Carolina and South Carolina primarily with longlines, Kali poles, and snapper reels during 1981–85 (n=359) and primarily with chevron traps during 1991–95 (n=114).

the bottom longline fishery developed. The decreases in 1984 and 1985 reflect a shift in effort to the pelagic longline fishery for swordfish (Low et al., 1987).

In South Carolina, the mean length of snowy grouper caught with longlines decreased steadily from 66 to 72 cm during 1983–84 to a low of 49 cm in 1996 (Fig. 13). No trend was evident in the length data for snowy grouper caught with snapper reels. The snowy grouper caught with snapper reels were consistently smaller than those caught with longlines because snapper reels were deployed in shallower water (Fig. 14).

Length data from North Carolina for snowy grouper caught with longlines showed a similar decreasing trend, though with greater interannual variation (Fig. 15). The mean length of snowy grouper caught with snapper reels has fluctuated, with peaks noted in 1985 and 1993.

Discussion

Status of the fishery

There are several indications that the snowy grouper population off the Carolinas is overfished: 1) size

at age of specimens caught with longlines and snapper reels has increased noticeably since the 1980s (Table 3), which could be a density-dependent population response to a decrease in competition for resources, 2) 81% of the specimens caught with longlines were ages 1–6, the majority (56%) of which were immature females (Tables 4 and 9), 3) the percentage of males appears to have decreased significantly, from 7% to 23% in the 1970s and 1980s to 1% in the 1990s (Table 7), 4) spawning stock ratio for the snowy grouper population in the South Atlantic Bight was 0.15 in the most recent assessment (SAFMC, 1993)—below the 0.30 level which means that the SAFMC considers the stock overfished, and 5) mean length of fish landed in the longline fishery has steadily decreased from 65 to 80 cm in the early 1980s to 50–60 cm in the mid-1990s (Figs. 13 and 15; see also Low, 1998).

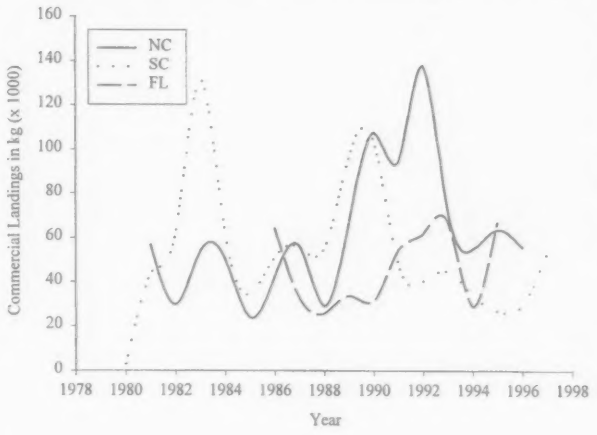


Figure 12

Commercial landings of snowy grouper in North Carolina, South Carolina, and Florida for all gear types, primarily longlines and snapper reels. Data were collected in individual states through their Trip Interview Program (TIP).

Snowy grouper are susceptible to rapid depletion in a localized area through fishing efforts. A study on a previously unexploited deep reef off North Carolina found that fishing can remove 3% of the reef population daily (Epperly and Dodrill, 1995). In less than three months, the catch per unit of effort and mean size of snowy grouper at that reef were reduced to levels comparable to other exploited sites. The mean size of snowy grouper landed in North and South Carolina during most of the 1990s (Figs. 13 and 15) is comparable to the size Epperly and Dodrill (1995) reported for exploited sites.

The increase in size at age over a ten-year period for fish from both gear types is noteworthy because this trend has been documented in populations that had experienced moderate to high levels of fishing mortality. Increases in size at age have been noted for silver hake (Helser and Almeida, 1997) and Atlantic halibut (Haug and Tjemsland, 1986) in the north Atlantic and several reef fish species (gag, red grouper, and red porgy) off the southeast coast of the United States and in the Gulf of Mexico (Johnson et al., 1993; Johnson and Collins, 1994; Harris and McGovern, 1997; Goodyear and Schirripa[3]). In our study, the increase in size at age may represent density-dependent growth in response to an increase in fishing mortality (Rothschild, 1986). Decreases in the abundance (Low, 1998) of co-occurring species on a similar trophic level, such as gray tilefish (*Caulolatilus microps*) and tilefish (*Lopholatilus chamaeleonticeps*), also may reduce competition for food and shelter in deepwater habitats. Snowy grouper and gray tilefish feed on macroinvertebrates, particularly crabs (Brachyura), and fishes closely associated with the substrate (Ross, 1982; Dodrill et al., 1993).

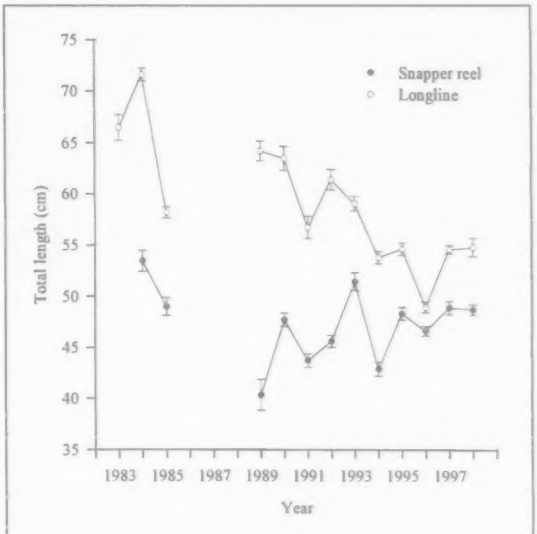


Figure 13

Mean length (±SE) of snowy grouper landed in South Carolina in the longline and snapper reel fisheries. Data were collected through the Trip Interview Program (TIP) at the South Carolina Department of Natural Resources. Sample sizes ranged from 22 to 851.

Density-dependent increases in growth rate generally indicate that a population or community is heav-

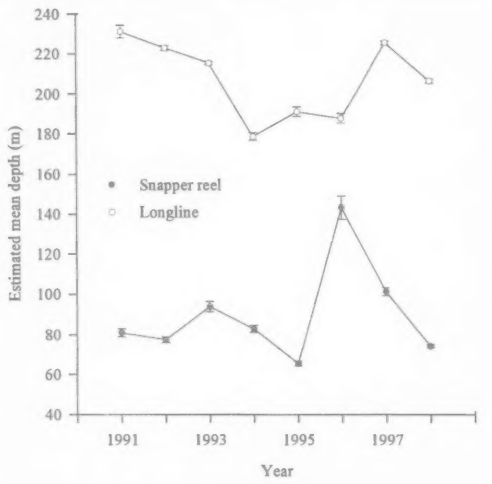


Figure 14

Estimated mean water depth (±SE), based on minimum and maximum depths provided by captains, during fishing efforts in the longline and snapper reel fisheries. Data were collected through the Trip Interview Program (TIP) at the South Carolina Department of Natural Resources from vessels landing their catches in South Carolina. Sample sizes ranged from 141 to 851.

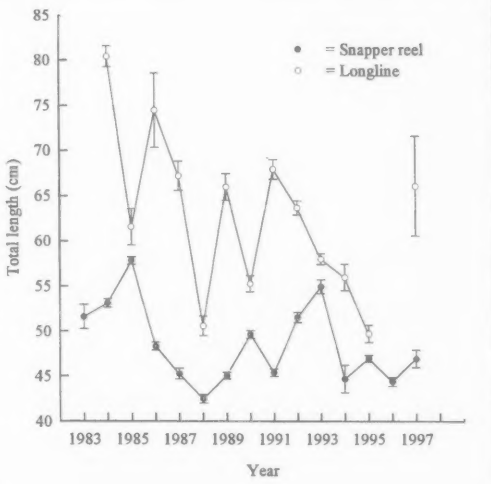


Figure 15

Mean length (±SE) of snowy grouper landed in North Carolina in the longline and snapper reel fisheries. Data were collected through the Trip Interview Program (TIP) at the North Carolina Department of Health, Environment, and Natural Resources. Sample sizes ranged from 5 to 1908 fish.

ily exploited and possibly overexploited. A situation of greater concern would be one where size at age has decreased after a sustained high level of fishing-induced mortality, as has been reported for red porgy, *Pagrus pagrus*, and vermilion snapper, *Rhomboplites aurorubens*, in our study region (Harris and McGovern, 1997; Zhao et al., 1997). There is evidence that faster-growing individuals in the populations of red porgy and vermilion snapper have been effectively eliminated, thus causing a decrease in size at age. Red porgy exhibited a density-dependent response after an initially high level of fishing mortality, but the sustained high level of mortality eventually removed the faster-growing individuals. Size at age should be monitored to ensure that this does not occur in the snowy grouper population.

The age composition of the snowy grouper landings also needs to be monitored because the longline fishery is presently supported by younger age classes (1–6). The present study showed that snowy grouper can attain an age of 29 yr, but only 19% of the fish caught on longlines were >age 6 (Table 4). The low percentage of older age classes in the landings supports the preliminary sex ratio data from the 1990s, showing that the percentage of males had significantly decreased.

Age and growth

A comprehensive comparison of growth data in our study with previously published results was not possible because 1) lack of large and old specimens and small sample sizes, and 2) differences in study area (Florida Keys in Moore and Labisky [1984]). One or more of these factors could explain the differences in size at age, k, and L_∞ between the results of two published studies (Matheson and Huntsman, 1984; Moore and Labisky, 1984) and our results for longline and snapper reel data from the 1980s. A primary reason for higher values of L_∞ in the published studies (Table 3) is that the growth curves do not exhibit asymptotes, which is probably due to low numbers of specimens greater than approximately 900 mm and older than 15–17 yr. In our study, all the data sets (2 bandit reel and 2 longline) had individuals over 1000 mm and at least 21 yr old. Sample sizes in two data sets were very small (<200): our longline or Kali pole data set (n=163) and the data set of back calculations (n=118) in Moore and Labisky (1984). Important factors that could not be evaluated on the basis of previous publications were 1) similarity of fishing gear, 2) method of increment interpretation, and 3) whether or not a weighting factor was used when fitting the von Bertalanffy growth model.

We feel confident that our assessment of the age structure in the snowy grouper population off the Carolinas was accurate, even though interpretation of growth increments was difficult and a minimal number of YOY specimens was available. The difficulty of assigning an age to the sagittae of snowy grouper had not been reported by other investigators, although it has been reported for other deepwater species of continental slopes. The clarity of the hyaline and opaque zones in otoliths (presumably sagittae) from hoki, *Macruronus novaezelandidae*, off New Zealand is highly variable and is divided into six categories based on internal features which are related to the ease of counting increments (Kuo and Tanaka, 1984). When otoliths are difficult to interpret, one option is to base population age structure only on the specimens for which age is easily assessed. Alternatively, ages can be estimated for nearly all specimens despite the difficulties, as we did in our study, with the assumption that the larger sample will represent the population better. We found that limiting the data set to only those specimens for which the difference in counts between readers was 0–1 increments did not improve the accuracy of the age-length key for specimens caught with longlines (Table 6). Thus, we advocate using the entire sample of specimens assigned an age. Crabtree and Bullock (1998) found that rejected otoliths tend to be from slower-growing older specimens, which could introduce bias into analyses. This bias was minimal in their study of growth in black grouper, *M. bonaci*, where each otolith was examined three times by two independent readers. Parameter estimates for the von Bertalanffy model based on all black grouper with ages were within one standard error of those based only on specimens for which the coefficient of variation of the six readings was ≤12%.

An important consideration in age determination is positive identification of the first annulus and any settling mark that may be deposited prior it. We believe that we have identified the first annulus because the largest YOY specimen (172 mm) had an estimated age of 191–291 days and the measurements of otolith radius for all YOY (n=3) were less than radial measurements to the first annulus in a subsample of 23 specimens that were age 1. Evidence to support our conclusion that these three specimens were YOY was found in another sampling effort, where six specimens 4–5 cm in length were caught with a trawl during August and September (Machowski[5]), the last two months of the spawning season. Moore and Labisky (1984) considered 150–175 mm specimens to be YOY, although they did not examine daily increments.

Validation of a technique for aging snowy grouper with otoliths has been weakly supported by previous studies. We found that marginal increments form annually and there is a peak in April and May that corresponds to the beginning of the spawning season. This finding concurs with the limited results of Matheson and Huntsman (1984) and Moore and Labisky (1984) who found that increment formation appeared to begin in April and peaked in June. Matheson and Huntsman (1984) measured marginal increments in 18 specimens collected during April through October and Moore and Labisky (1984) examined specimens collected during March through July (n not reported). Waltz[4] found a wider period of increment formation, April through September, although he was not able to conclude that increments form annually because samples were lacking for four months.

Reproduction

The reproductive pattern of snowy grouper needs to be investigated more comprehensively and the sex ratio should be assessed again, given the small sample size in 1993–94 (n=82), because there is evidence that reef fish species, particularly grouper, which change sex and aggregate to spawn, are more susceptible to size-selective mortality and overexploitation (Bannerot, 1987; Huntsman and Schaaf, 1994; Coleman et al., 1996). The capture of only one male in the 1993–94 samples, which appeared to be representative of the population based on commercial landings, is reason for concern because the percentage of males has apparently decreased from the 7–23% for samples collected with three gear types in the 1970s and 1980s (Table 7).

Large decreases in the number of males have been documented for two other grouper species in the southeast region. Percentages of males in populations of gag and scamp in the Gulf of Mexico, groupers that are known to form small spawning (10's to 100's of individuals) aggregations, decreased from 17% to 1% and 38% to 18%, respectively, between the 1970s and early 1990s (Coleman et al., 1996). A similar decrease, from 20% to 6%, was noted for gag along the Atlantic coast of the southeastern United States during the same period (McGovern et al., 1998). The resultant decrease in genetic diversity has been documented for gag (Chapman et al., 1999), and its ramifications are currently of great concern to many fishery scientists in the southeast region.

The size (767–1090 mm) and age (8–29 yr) of 97 male specimens in the present study and the capture

[5] Machowski, D. 1998. S. Carolina Department of Natural Resources, P.O. Box 12559, Charleston, SC, 29422. Personal commun.

of two specimens in the process of changing from female to male is conclusive evidence that snowy grouper are protogynous hermaphrodites. Moore and Labisky (1984) reported males as young as age 6 and some males with evidence of recent sex change. It is likely that we collected only two transitional specimens because sex change occurs after a female finishes spawning, during months when sample sizes in our study were small. Sex change in other grouper species, gag for example, occurs primarily during the first two to three months after the spawning season (McGovern et al., 1998), before males and females become spatially separated (Coleman et al., 1996).

Our findings on age at maturity and spawning season are in general agreement with the results of Moore and Labisky (1984) for snowy grouper in the Florida Keys. They found that the smallest mature female and largest immature female were age 3 and age 5, respectively, whereas the smallest mature female from 1980 to 1985 in our study was also age 3, but small percentages (<10%) of the age 7–9 females were immature (Table 9). Females and males in the Florida Keys were in spawning condition from April through July, although no mature fish were sampled in other months (Moore and Labisky, 1984). We found that females off the Carolinas spawn from April through September—possibly longer owing to small sample sizes in October through March.

The capture of 1160 specimens, some of which were assessed macroscopically as spawning, in four trawl collections on the exploratory squid cruise in June 1978 suggests that snowy grouper may form spawning aggregations. Estimates of density would have been much higher than 13.5–79.5 kg/ha calculated from the trawl data if the fish were caught in only a small part of the area sampled during tows of 7.4–18.5 km. Dodrill and Epperly (1995) reported that the initial density of exploitable snowy grouper on a 2700-m^2 virgin reef off North Carolina was 11 kg/m^2.

Depth distribution

Fishery-independent data collected over several years and with various gear types show that fish length is positively correlated with water depth (Fig. 11). Longlines, Kali poles, and snapper reels were the primary gear types deployed in waters >150 m and the waters <100 m were sampled primarily with chevron traps. Chevron traps are not known to be selective for snowy grouper <600 mm. Dodrill et al. (1993) speculated that the low abundance of adults in shallow waters may in part reflect years of intensive fishing pressure in 40–120 m depths and only relative recent fishing activity at depths >183 m off North Carolina. Alternatively, we propose that snowy grouper may migrate to deeper water toward the end of the juvenile stage.

Fish length and water depth data from the commercial fisheries (Figs. 13 and 14) concurred with the positive correlation noted in the Florida Keys (Moore and Labisky, 1984) and off Georgia and South Carolina (Low and Ulrich, 1983), although depth data from fishermen may be less accurate than fishery-independent data. We found small juveniles in shallow water, a finding that agrees with the results of Moore and Labisky (1984) and with observations from submersible dives that documented juvenile snowy grouper (<300 mm) between 46 and 91 m, but not in deeper waters (Parker[6]).

Accurate assessment of population parameters requires knowledge of juvenile and adult distributions of snowy grouper as well as characteristics of the fishery. The snapper reel fishery catches a greater proportion of younger age classes than does the longline fishery because fishing efforts are generally restricted to areas <100 m in depth (Figs. 13 and 14). The longline fishery presently catches a greater proportion of older age classes than does the snapper reel fishery because regulations established by the SAFMC restrict longlines to waters deeper than 91 m.

Conclusions

As other investigators have suggested, rebuilding grouper populations may require a novel approach such as long-term area closures or individual transferable quotas (Epperly and Dodrill, 1995; Coleman et al., 1996). At present, the regulations enacted to rebuild the snowy grouper population include an annual quota of 245,082 kg, with a trip limit of 1134 kg (SAFMC, 1993). Traditional management measures such as minimum size limits will not be effective because snowy grouper experience fatal embolisms while being brought to the surface from deep waters (Matheson and Huntsman, 1984). Future research should focus on improving our understanding of reproductive pattern (e.g. spawning behavior, spatial and temporal aspects of distribution) and include a thorough assessment of sex ratio and an updated assessment of population age structure.

Acknowledgments

We thank F. Rhode, J. Francesconi, and other assisting members of the North Carolina Department of Health,

[6] Parker, R., Jr. 1997. National Marine Fisheries Service, 101 Pivers Island Rd., Beaufort, NC, 28516. Personal commun.

Environment and Natural Resources for obtaining otoliths and gonads from snowy grouper in North Carolina. In South Carolina, the personnel of the MARMAP program of the S.C. Department of Natural Resources assisted with port sampling. Captains D. Juel, S. Juel, J. Murray, S. Shelley, and the late J. D. Skipper III let us remove otoliths from their catches of snowy grouper and brought in whole specimens for the study of reproductive biology. We very much regret the loss of Captain Skipper, his vessel, and a crew member. C. Jackson and T. Prince of the Southport Fish Market, and F. McGinn and R. McGinn of the Little River Fish House, allowed us to process specimens at their businesses. R. Dixon and G. Huntsman provided gonad samples collected by the National Marine Fisheries Service Beaufort Laboratory. K. Grimball and O. Pashuk prepared the histological sections. B. Zhao generated the parameter estimates for the von Bertalanffy model. T. Azarovitz, T. Reisinger, and D. Machowski contributed to the retrieval of data from the 1978 squid cruise originally collected by E. Gutherz. Earlier drafts of the manuscript were examined by P. Harris, J. McGovern, and three anonymous reviewers. This project was funded through the National Marine Fisheries Service MARFIN grant NA37FF0046-01 and the MARMAP contract 50WCNF006002.

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Notes on the biology of *Cephalurus cephalus* and *Parmaturus xaniurus* (Chondrichthyes: Scyliorhinidae) from the west coast of Baja California Sur, México

Eduardo F. Balart
División de Biología Marina, Centro de Investigaciones Biológicas del Noroeste, S.C.
Apdo. Postal 128, La Paz, B.C.S., México 23000
E-mail address: ebalart@cibnor.mx

Jeanette González-García
Carlos Villavicencio-Garayzar
Laboratorio de Elasmobranquios, Universidad Autónoma de Baja California Sur
Apdo. Postal 19-B, La Paz, B.C.S., México 23000

The head, or lollipop, shark *(Cephalurus cephalus)* and filetail catshark *(Parmaturus xaniurus)* are found in the eastern Pacific in waters ranging from 245 to 828 m and from 91 to 1251 m depth, respectively (Castro, 1983). Little is known of their biology because they are rarely captured. *Cephalurus cephalus* is a benthic catshark and has been recorded from southern California, Gulf of California, the Revillagigedo Archipelago, and off the coasts of Peru and Chile (Kato et al., 1967; Meléndez and Meneses, 1989; Pequeño, 1989). Mathews and Ruíz (1974) reported it from the upper Gulf of California, México, and Castro-Aguirre (1981) gave basic information about its morphology, taxonomy, and ecology based on 11 specimens from this locale.

Parmaturus xaniurus is distributed from central California to the Gulf of California (Castro, 1983). Cross (1988) has described its general biology and Lee (1969) reported that, in the Santa Barbara basin, it consumed myctophids. Cailliet[1] concluded that juveniles were mesopelagic, adults demersal, and noted that efforts to determine their age have failed because of the absence of annual rings on the vertebrae.

The objective of this study was to provide additional data on the biology of *C. cephalus* and *P. xaniurus*, with information on length-weight relationships, and notes on reproductive biology, including the sizes of oocytes and embryos, the sex ratio of embryos, and clasper length, for specimens from the west coast of Baja California Sur, México.

Materials and methods

Fish were sampled by bottom trawling with commercial shrimp nets (20-m mouth, 30-mm mesh size) during the oceanographic expedition EP9505 along the Pacific coast of Baja California Sur, México. Trawls were made at depths of 50–280 m from the RV *El Puma* during May 1995. On 5 May two scyliorhinid species (*C. cephalus* and *P. xaniurus*) were collected and whole specimens were frozen. All specimens were captured at the following three stations: 26°01.9' N, 113°26.9'W (280 m); 25°59.6'N, 113°20.5'W (260 m); 26°00.7'N, 113° 18.3'W (230 m). Bottom temperature at the collection sites was 10.0°C. Total weight (W, g), and total length (TL, mm) were recorded. Females were dissected, the diameter of all visible oocytes were recorded, and embryos were removed. For the subsequent analysis of *C. cephalus*, previously published data were also used. The length-weight relationship was determined with STATISTICA software (StatSoft, Inc., 1995) by using the exponential function of the form

$$W = aTL^b,$$

as used by Cross (1988) for the filetail catshark. Length-weight relationships were compared between males and females by using an analysis of covariance.

Results and discussion

Seventy-five catsharks were obtained: 51 *P. xaniurus* and 24 *C. cephalus*. The latter species is recorded for the first time along the west coast of the Baja California peninsula. At one station, *C. cephalus* was captured together with *P. xaniurus*, as reported by Mathews and Ruiz (1974). Other organisms collected at the three stations were the fishes *Merluccius angustimanus*, *Physiculus rastrelliger*, *Kathetostoma averruncus*, *Synodus lucioceps*, *Hippoglossina stomata*, and *Lepophidium* spp., and the crustaceans *Pleuroncodes planipes* and *Cancer johngarthii*.

Cephalurus cephalus

Of the 24 specimens, 23 were female, with a range of 224 and 295 mm TL ($\bar{x}$=249 mm TL). The single male specimen was 298 mm TL. Previously reported maximum total

[1] Cailliet, G. 1981. Ontogenetic changes in the depth distribution and feeding habits of two deep-dwelling demersal fishes of California: sablefish and filetail cat sharks. Paper presented at the sixty-first annual meeting of the Am. Soc. Ichtyol. Herpetol.

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Fish. Bull. 98:219–221 (2000).

lengths for females and males were 295 and 245 mm TL respectively (Castro-Aguirre, 1981). The length-weight relationship suggested allometric growth and was described by the following equation

$$W = 0.000012\ TL^{2.84} \quad \text{(Fig. 1A)}.$$

The b coefficient was not significantly different ($F_{1,36}$= 2.15) between the sexes.

Females of this species have two functional ovaries and oviducts. Sixteen (70%) of the females had mature oocytes in their ovaries and a mean oocyte diameter of 10.5 mm (range 2.0–21.8 mm). The relationship of mean oocyte diameter with TL indicated that all females were mature (Fig. 1B). The size at first maturity has previously been estimated to be 190 mm TL (Compagno, 1984). The large diameter of the oocytes suggested that spawning occurs in early summer.

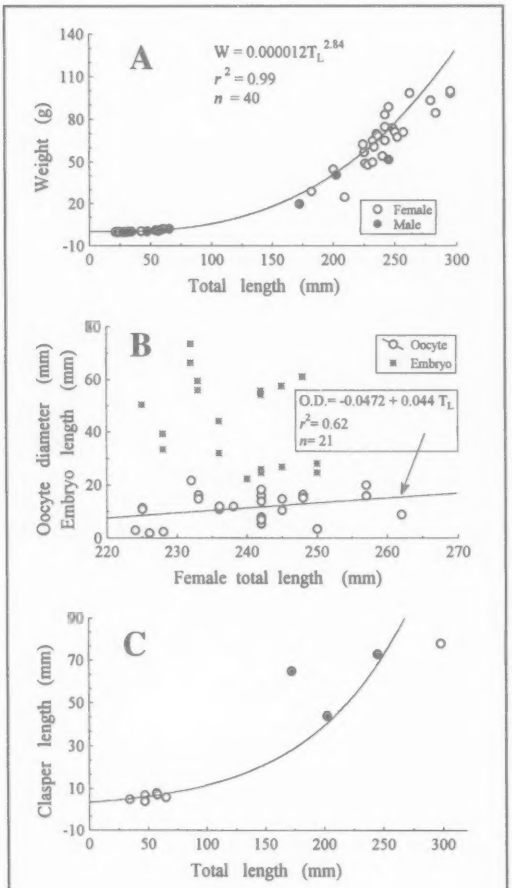

Figure 1

Cephalurus cephalus. (**A**) Length-weight relationship. (**B**) Relationships between oocyte diameter and embryo total length with female total lengths. (**C**) Relationship between total length and clasper length. Data less than 75 mm TL are from embryonic material. Data from Castro-Aguirre (1981) are denoted by solid circles.

Embryonic development of *C. cephalus* occurs in the uterine tract and is described as aplacental viviparity or ovoviviparity (Wourms, 1977, 1981; Compagno, 1984). The thin-walled egg cases of *C. cephalus*, which are retained in the oviducts, were observed in 11 individuals. The three largest females had neither eggs in the oviducts nor large oocytes in their ovaries and may have finished their reproductive cycle.

The mean size of the 19 embryos was 43 mm TL (size range 21 to 65 mm TL), which is less than the estimated size at birth (100 mm; Compagno, 1984), and the sex ratio of embryos (9 males, 10 females) was aproximately equal. The relation between clasper length and total length of adults and embryos is illustrated in Figure 1C.

Parmaturus xaniurus

Of 51 specimens captured, 22 were female and 29 were male. Females measured 108 to 350 mm TL ($\bar{x}$=184 mm TL), males 117 to 380 mm TL ($\bar{x}$=233 mm TL). The largest recorded lengths are 574 mm TL for females and 600 mm TL for males (Springer, 1979). The length-weight relationship of *P. xaniurus* indicated allometric growth (Fig. 2A) and was described by the following equation:

$$W = 5.5 \times 10^{-7}\ TL^{3.34}.$$

The analysis by sex (males: W= 6.76×10^{-7} $TL^{3.31}$; females: W=9.53×10^{-7} $TL^{3.22}$) did not reveal significant differences in the b coefficient ($F_{1,45}$=0.14) as reported by Cross (1988) for specimens from the upper continental slope off southern California.

All females examined were immature and did not contain mature oocytes. Cross (1988) noted that females first reached maturity at 425–475 mm TL in southern California waters. *Parmaturus xaniurus* is oviparous, and the egg cases of this species have been described by Cox (1963). Embryonic development lasts approximately one year (Compagno, 1984; Cross, 1988). The relationship between total length and clasper length suggested that males matured at 340 mm TL (Fig. 2B), and this size at first maturity was smaller than that previously recorded (375 to 425 mm TL; Cross, 1988). This may reflect latitudinal differences in the reproductive biology of *P. xaniurus*.

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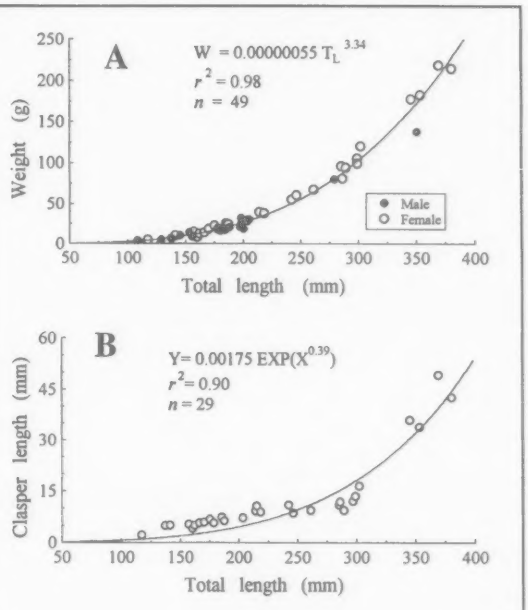

Figure 2

Parmaturus xaniurus. (**A**) Length-weight relationship. (**B**) Relationship between total length and clasper length.

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Penaeid shrimp landings in the upper Gulf of California in relation to Colorado River freshwater discharge

Manuel S. Galindo-Bect
Instituto de Investigaciones Oceanologicas
Universidad Autonoma de Baja California
Km 103 carretera Tijuana-Ensenada
Ensenada, Baja California, México

Edward P. Glenn
Environmental Research Laboratory
2601 East Airport Drive
Tucson, Arizona 85706
E-mail address (for E. P. Glenn, contact author): eglenn@ag.arizona.edu

Henry M. Page[1]
Kevin Fitzsimmons[2]
Luis A. Galindo-Bect[3]
Jose M. Hernandez-Ayon[3]
Robert L. Petty[1]
Jaqueline Garcia-Hernandez[2]
David Moore[2]

A commercial trawl fishery in the upper Gulf of California provides the principle source of income for the coastal communities of the region, but catches of estuarine-dependent crustaceans and fish have declined in recent years (Hernan, 1997; Cudney-Bueno and Turk-Boyer, 1998). Declines in shrimp landings, mainly *Litopenaeus stylirostris* (formerly classified as *Penaeus stylirostris*) (Perez-Farfante and Kinsley, 1997) have been attributed primarily to over-exploitation of the resource and to viral diseases (Rosas-Cota et al., 1996; Hernan, 1997).

The Biosphere Reserve of the upper Gulf of California and Colorado River Delta was created in 1993 to address some fisheries management problems. A more fundamental problem, however, may be the lack of river flow after construction of upstream dams. Historic reductions in river discharge have caused dramatic increases in salinity in the estuary and changes in the distribution of nutrients (Alvarez-Borrego et al., 1975; Hernandez-Ayon et al., 1993). Since 1979, occasional flood releases have entered the upper Gulf of California by means of the Colorado River when upstream impoundments are filled (Glenn et al., 1996).

Effects of freshwater on penaeid shrimp population development are controversial (Garcia and Le Reste, 1981; Day et al., 1989), but recruitment of spawning stocks of white shrimp (*Penaeus setiferus*) has been positively correlated with river discharge in the southwestern Gulf of Mexico and has been attributed to an expansion in estuarine nursery habitat for white shrimp (Garcia, 1991). River discharge also can stimulate the migration of subadults from estuaries (Deben et al., 1990; Vance et al., 1998). Fishermen have a strong perception that shrimp and fish catches in the northern Gulf of California are related to freshwater discharge from the Colorado River (Cudney-Bueno and Turk-Boyer, 1998). To evaluate their perception we conducted a correlation analysis of shrimp landings at San Felipe Baja California (nearest shrimping station to the delta) with freshwater discharges from the Colorado River to the northern Gulf of California.

Materials and methods

Data on annual shrimp landings and number of trawlers legally fishing from San Felipe were obtained from the Secretary of Environment, Natural Resources and Fish (SEMARNAP), San Felipe, Mexico. Landings were available from 1977 and number of trawlers from 1982. The artisanal catches by small boats (pangas) or the significant illegal shrimp fishery are not accounted for in reported shrimp landings. Annual shrimp landings serve as indicators of the variability in the total landings and are reported for all species of shrimp, even though landings are >90% *L. stylirostris* in San Felipe (Rosas-Cota et al., 1996). Data on freshwater discharge of the Colorado River were from the Southerly International Border (S.I.B.) gauging station which is below the last diversion on the river and were obtained from the United States

[1] Marine Science Institute
University of California
Santa Barbara, California 93106
[2] Environmental Research Laboratory
2601 East Airport Drive
Tucson, Arizona 85706
[3] Instituto de Investigaciones Oceanologicas
Universidad Autonoma de Baja California
Km 103 carretera Tijuana-Ensenada
Ensenada, Baja California, México

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Bureau of Reclamation, Yuma, Arizona (Williams[1]). We assumed that this flow entered the delta and the upper Gulf of California.

Annual shrimp landings and landings divided by numbers of trawlers (normal catch per unit of effort, CPUE) were correlated with river flow and number of trawlers. Our normal CPUE was a crude approximation of stock abundance or catchability. We lacked actual fishing time (days, weeks, hours of net deployment), size frequency of the legal vessels, and number of small boats (pangas) fishing. We made landings lag river discharge by one year because the life cycle of shrimp from hatching to capture is approximately one year (Gracia-Pamanes[2]). Transformed river flow ($\log_{10}$) was tested for nonlinearity; then we conducted a multiple regression analysis to predict shrimp landings from variables that were individually correlated ($P<0.05$) with landings.

Results

Annual shrimp landings ranged from 701 metric tons (t) (1983–84) to 217 t (1992–93), decreasing significantly from 1977 to 1996 ($r=0.78$, $P<0.001$, Fig. 1A). The reported number of trawlers legally fishing from San Felipe ranged from a high of 59 in 1988 to a low of 20 in 1995 (Fig. 1B). Catch per unit of effort (CPUE) increased from 1982 to 1984, then markedly decreased back to the 1982–83 level in 1985, remaining low until 1993, after which a positive trend was achieved and the highest CPUE ever was recorded in 1995 (Fig. 1C). There were substantial flows (>700 million cubic meters, Mm^3) in 8 of the 21 years from 1976 to1996 and varied over 10^4-fold, ranging from 1 Mm^3 in 1990 and 1996 to 15,657 Mm^3 in 1984 (Fig. 1D). Highest volume occurred between 1980 and 1987 as a result of overflow from Lake Powell in the United States (Glenn et al., 1996). The flow spike in 1993 was due to releases from Painted Rock Dam on the Gila River in Arizona. Periods of significant river flow at the S.I.B. were closely matched to El Nino Southern Oscillation (ENSO) events that occurred in 1983 and 1993.

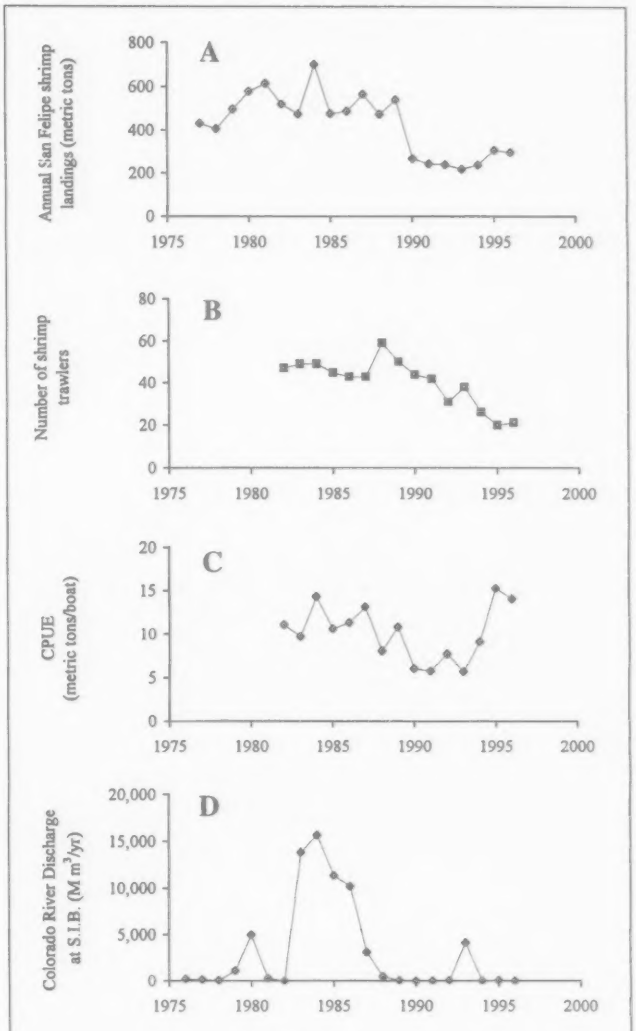

Figure 1

(**A**) Annual San Felipe shrimp landings (metric tons); (**B**) Number of shrimp trawlers fishing annually in San Felipe; (**C**) CPUE (metric tons/boat); (**D**) Colorado River freshwater discharge flow below the Southerly International Border (million m^3/yr).

Shrimp landings were significantly ($P<0.05$) correlated with same year river discharge, but $\log_{10}$-transformed river discharge in the year prior to shrimp harvest produced the highest correlation coefficient ($r=0.67$, $P<0.001$) (Table 1). The number of trawlers also significantly correlated with shrimp landings ($r=0.77$, $P<0.001$), as expected. The best correlation (r) of shrimp landings was the product

[1] Williams, B. 1998. United States Bureau of Reclamation, Yuma, Arizona. Personal commun.

[2] Garcia-Pamanes, F. C. 1992. Biologia reproductiva y dinamica poblacional del camaron azul Penaeus stylirostris en el Alto Golfo de California. Instituto de Investigaciones Oceanologicas, Universidad Autonoma de Baja California, Ensenada. Unpubl. final report.

Table 1

Correlation coefficients (r) and significance levels (P) from regression analysis relating San Felipe annual shrimp landings (1977–96) and CPUE (1982–96) to rainfall and discharge of the Colorado River at the Southern International Border. A "1-year lag" indicates that shrimp landings were paired with the previous year's river discharge in the correlation analysis.

	Correlation with shrimp landings		Correlation with CPUE	
Variable	r	P	r	P
River discharge	0.47	0.0362	0.25	0.3360
Log_{10} river discharge	0.54	0.0112	0.25	0.3368
River discharge (1-yr lag)	0.52	0.0127	0.34	0.1826
Log_{10} of river discharge (1-yr lag)	0.67	0.0006	0.38	0.1304
Number of shrimp trawlers	0.77	0.0003	0.18	0.4804
Log_{10} of river discharge (1-yr lag) × number of shrimp trawlers	0.80	0.0004	0.29	0.8771

of log_{10}-lagged river discharge and number of trawlers (r=0.80, P<0.001). CPUE was not significantly (P>0.05) correlated with river flow or number of trawlers (Table 1), nor with total landings (r=–0.26, P=0.31). The equation of best fit (0.64) for predicting shrimp landings took the form

$$Y = a + m(X_1X_2),$$

where X_1 = log_{10}-lagged river discharge (Mm^3/yr);
X_2 = number of trawlers;
Y = shrimp landings (t/yr);
M = the slope of the equation (1.67); and
a = the Y-intercept (232 t/yr).

Discussion

Our analyses represent a first attempt to identify relationships between variability in shrimp landings in the upper Gulf of California and factors influencing these landings. Total shrimp landings and the size of the shrimping fleet at San Felipe have declined over the past 15 years. Social and economic changes have affected shrimping. In the late 70s and early 80s shrimping was reserved for social units (cooperatives), with the result that privately owned shrimp trawlers were banned from the fishery. In addition, the government subsidized building of additional vessels and many new unskilled fishermen entered the industry. Then policies were reversed in the late 1980s, private boats returned, interest rates increased, and many of the shrimp trawlers were removed from the fleet.

We found a significant relationship (P<0.001) between total catch and the rate of freshwater discharge of Colorado River water into the marine ecosystem, although the mechanisms through which river discharge might affect the shrimp fishery are unknown. Lower salinity may improve the survival of early life stages by providing "enlarged nursery" protected habitat (Garcia, 1991), even though *L. stylirostris* and *P. californiensis* are generally considered euryhaline species (Hernan, 1997), having large numbers of postlarvae and juveniles in hypersaline habitats (Brusca, 1980; Page[3]). Salinity and nutrient gradients in the estuary and upper Gulf during river flows have not been reported to our knowledge.

Future plans for the Colorado River will likely decrease freshwater discharge into the estuary as more water is diverted upstream for farms and domestic use (Morrison et al., 1996). Our analyses suggest that decreases in river discharge to the delta and estuary may adversely affect shrimp landings. The United States and Mexican governments should initiate a research program on the effects of river flow on ecologically and commercially important species in the upper Gulf of California and incorporate these findings into a comprehensive management plan for the Biosphere Reserve as well as the Colorado River Basin at large.

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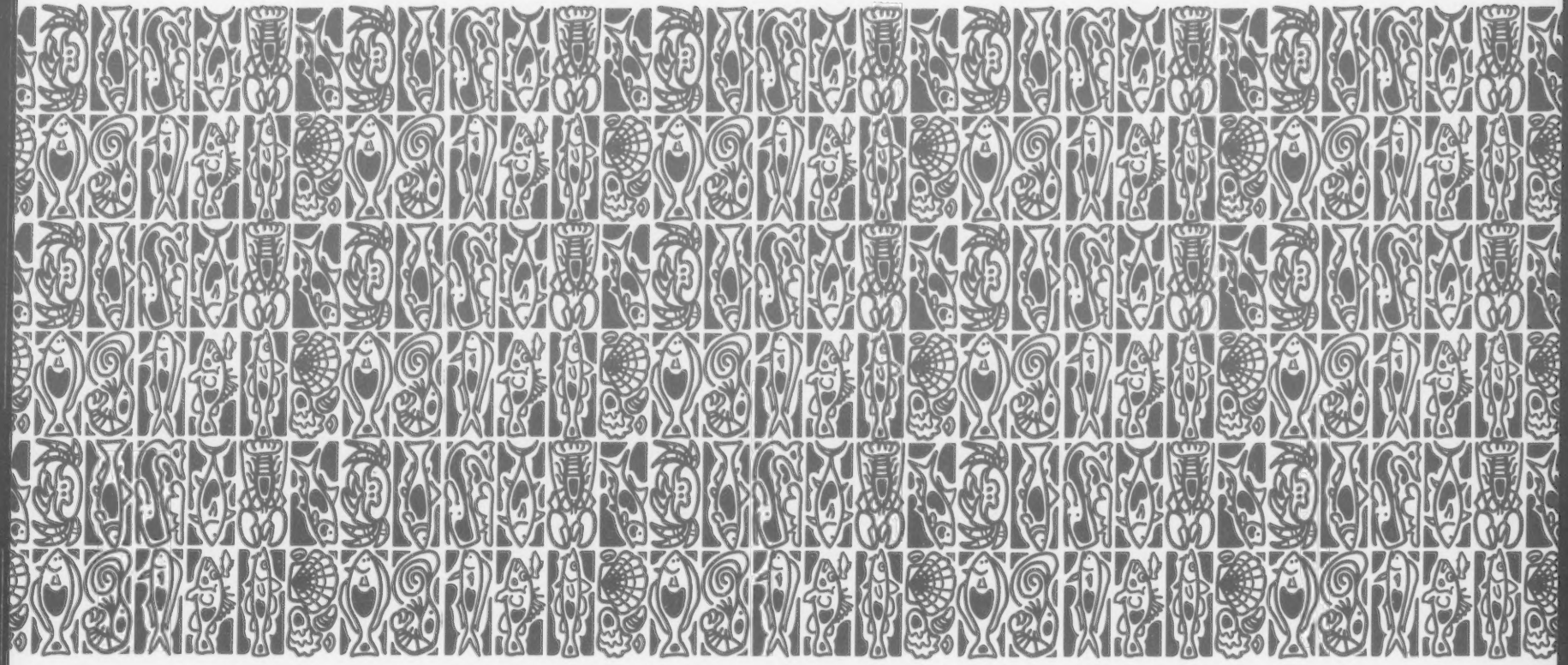
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